STUDIES ON RAT SERUM AMYLASE

George Stuart Wiberg B.Sc, M.Sc.

1955

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ABSTRACT

The salivary and pancreatic amylases of the rat are responsible for the digestion of starch in the stomach and small intestine. However, the amylase found in the blood serum of this animal is of unknown origin and significance. The present investigation is concerned with a study of this serum enzyme, with special reference to the factors which control its level.

It was found that food was the most important of these factors. A positive correlation was observed between food intake and serum amylase activity. This effect was modified by the dietary concentration of carbohydrate, fat, or protein. Several other agents which definitely influenced rat serum amylase were shown to be related to food consumption. These included: age, sex, sex hormones, removal of the hypophysis, and administration of crystalline pituitary growth hormone to the hypophysectomized animals. An exceptional situation was noted in alloxan diabetic animals, where destruction of pancreatic islet cells was accompanied by polyphagia, but serum amylase activity was unaffected.

Ablation of the pancreas lowered the level of the serum amylase. This indicated that the pancreas is a primary source of the enzyme. Extirpation of the gonads, or partial hepatectomy produced only a temporary diminution of enzyme values and this could be attributed to surgical trauma.

An examination of the body tissues that are high in amylase activity revealed that the intestinal mucosa and the depot fat may also contribute to serum amylase activity. The parotid glands possessed the highest amylase content of any tissue investigated, yet in studies where

the serum amylase was elevated or depressed, enzyme level of parotid tissue remained unaltered.

The injection of codeine elevated serum amylase levels in the mouse but not in the rat. This response is dependent upon the presence of a gall-bladder; an organ which is lacking in the rat. Intraperitoneal administration of ethionine produced marked diminution of the amylolytic activity of rat serum. Most of this decrease could be ascribed to anorexia, but a significant proportion could be attributed to profoundly depressed levels of pancreatic amylase, which accompanies destruction of acinar tissue. On the other hand the enzyme level of the parotid glands was unaffected by ethionine injection.

It would appear from this investigation that the chief source of serum amylase in the rat is the pancreas.

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THE UNIVERSITY OF ALBERTA

STUDIES

ON RAT SERUM AMYLASE

A DISSERTATION

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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GENERAL INTRODUCTION

I. A. Historical

In 1811, Kirchoff (1) reported that wheat extracts possessed a digestive action upon starch; this is now regarded as the first published description of an enzyme. A similar effect was found in human saliva by Leuchs (2) in 1831. Dubrunfaut in 1830 (3) prepared an extract of malt which converted starch into sugar. Three years later, in 1833, Payen and Persoz (4) discovered that the addition of alcohol to such malt extracts resulted in the precipitation of a substance which could be dried and preserved, and which had a very powerful action upon starch. This substance they called diastase.

Amylase is now known to have a ubiquitous distribution throughout the animal, plant, and microbiological worlds. This wide distribution, together with its important role in the economy of the animal, plant, and bacterium, has led to its extensive investigation by many workers.

Much of this early work has led to conflicting reports as to the action of this enzyme. Briefly, the action of amylase on starch is accompanied by certain changes, viz., an increase in reducing power, a decrease in the viscosity of the starch paste, and a decrease in the intensity and quality of the color of the starch-iodine complex with its eventual disappearance. Amylase preparations from different sources produced these changes to varying degrees. In some cases, the decrease in viscosity and loss of the starch-iodine color were the most pronounced, in other cases, the increase in reducing power predominated.

The final answer to these anomalies lay in the discovery that two different types of amylases existed, the α and β amylases. The

former produce a rapid drop in the viscosity of starch paste and marked decrease in the starch-iodine color before considerable increase in reducing power has taken place. The latter group produce a considerable increase in the reducing sugar before a noticeable loss of viscosity or disappearance of the starch-iodine color appears.

Along with the discrepancies of behaviour of various amylase preparations, it was also found that different sources of starch gave divergent results from the standpoint of the starch-iodine color. Concerted research on the structure of starch has led to the conclusion that it is a heterogeneous mixture of two components now known as amylose and amylopectin.

B. Constitution of Starch

As was noted, starch is now considered to consist of two fractions, amylose and amylopectin. In general, it might be said that the latter fraction is present in the greater amount, but the relative percentage of the two polysaccharides does vary with the species of the plant.

Amylose is a linear polymer of α-glucopyranose, joined by α,1-4- linkages. It consists of from 200 to 300 units, and has a molecular weight of the order of 50,000. Amylose prepared from different species differs in chain length, e.g., potato amylose has a molecular weight of from 15,000 to 100,000, while the amylose of seeds may contain compounds with molecular weights of 400,000. Amylose gives a deep blue color with iodine.

Amylopectin consists of shorter chains of from 25 to 45 linear α,1-4- glucopyranose linkages. These chains are bound with α,1-6- cross linkages, which appear to be about 12 units in length. A highly ramified structure is thus found. Amylopectin molecules are much larger than amylose. The molecular weight varies from 50,000 to 1,000,000. Amylopectin gives a violet to brown color with iodine.

C. Mode of Action of the Amylases

As the α and β amylases produce different results with respect to their enzymic degradation of starch, it is probable that their point of attack and method of attack upon the substrate is also different. Both amylose and amylopectin are susceptible to attack by α and β amylase.

β amylase has the simplest type of action and will be discussed first. It attacks the substrate from the non-reducing end and splits off successive maltose fragments. In a linear polymer such as amylose it is possible to secure 100% hydrolysis in theory. In amylopectin, it also attacks from the non-reducing end, and works down the chain removing successive maltose fragments until it reaches a point of bifurcation, i.e., the 1-6 glucosidic bond. Since it is unable to effect cleavage, enzymic action ceases at this point. In branched substrates, such as amylopectin and glycogen, hydrolysis can only proceed to 50-60% of the theoretical maximum calculated as maltose.

The action of α amylase is quite dissimilar. The enzyme attacks the substrate from deep within the molecule producing large fragments known as dextrins. These dextrins are successively broken down into smaller and smaller units until they emerge into the oligosaccharide class. The viscosity of starch paste and the starch-iodine color complex are both colloidal properties, and as the size of the starch particle decreases these properties will likewise tend to diminish. The cleavage takes place at 1-4 linkages but it is not known whether the attack is randomized or is to some extent dictated by the configuration of the substrate. Maltose and glucose appear as hydrolytic products in the latter stages of the amylolysis. The time and extent of their appearance depend to a large extent upon the conditions of the incubation.

The α amylases require chloride ions for their activity whereas the β amylases do not. Other anions may replace the chloride ions, e.g., bromide and nitrate anions.

Ohlsson (5,6) proposed that the α amylase be called dextrinizing amylase from its obvious dextrinizing effect and that the β amylase be called saccharifying amylase since it produced only reducing sugars. Another terminology has been introduced by Kuhn (7, 8) based on his observations of the optical rotation during amylolysis. The α amylases produce sugars in their optical α form, whereas the β amylases produce sugars in the β form. Kuhn suggested the terms α and β amylases. The former are the dextrinizing amylases and the latter are the saccharifying amylases. Generally speaking, plant and bacterial amylases are of both the α and β variety, while animal amylases are restricted to the α type.

SERUM AMYLASE

I. Introduction

The presence of amylolytic activity in human blood was first described by Magendie in 1846 (9). Serum amylase has since been found to be a normal constituent of mammalian blood in general and the horse, ox, sheep, goat, cat, dog, rat, and mouse, as well as man, have all been the subjects of special study. Carlson and Luckhardt (10) have made an extensive investigation of the distribution of this enzyme amongst the different species.

The origin of this enzyme has long interested investigators. The pancreas, liver, and the salivary glands are the most frequently postulated sources. Somogyi (11) in a critical review of the literature up to 1941, concluded that the source of serum amylase is unknown.

Attempts to crystallize serum amylase have so far been unsuccessful, although both human pancreatic (12) and human salivary amylase (13) have been crystallized as well as that of swine pancreas (14). Surgenor,Hunter and Brown (15) give an account of an attempt to crystallize the serum enzyme. Although a 1700-fold increase in activity was achieved, it still possessed but 0.1% of the activity of crystalline amylase isolated from tissue.

The serum amylase of rats has been but slightly investigated and as a consequence little is known as to the physiological factors which control its level. This investigation is concerned with the study of the normal levels of serum amylase in the rat and the effect of sex, age, diet, hormones, and other physiological factors on the maintenance of these levels. The role of certain organs in the economy of rat serum

amylase has also been evaluated. In addition, the body tissues have been examined for amylase content. Those tissues, which were rich in amylolytic activity, were the objects of further study to determine if they could be assigned any part in the supply of amylase to the blood stream.

II. Experimental

A. The Estimation of Amylase Activity

(1). Methods Available

The methods described in the literature for the determination of amylase activity are numerous. They are usually based on one of the following changes accompanying amylolysis:

1. increase in reducing sugar,
2. change in the starch-iodine color, and
3. decrease in viscosity.

(a) Saccharogenic Techniques

The enzyme preparation is incubated along with substrate and buffer under a given set of conditions. An aliquot is removed and the amount of reducing sugar is estimated as maltose or glucose. These methods differ in the technique of sugar determination. Besides the older alkaline copper, iodometric, and ferricyanide methods, newer techniques have been developed which use dinitrosalicylic acid, anthrone, or picric acid.

The principal objection to these methods is that they are not specific in the measurement of amylase activity. Certain tissues, e.g., liver and muscle, contain phosphorylase, which also attacks starch, in the presence of phosphate, with the liberation of glucose-1-phosphate. Tissue phosphatases hydrolyze the glucose-1-phosphate to glucose and inorganic phosphate. Consequently, assay of tissues, and especially liver and muscle, for amylase will yield high results, unless a phosphorylase inhibitor is also included in the incubation mixture.

(b) Dextrinogenic Techniques

The starch-iodine color complex has been the basis of many methods for estimation of amylase activity. The Wohlgemuth method (16) has been used extensively in Europe and North America. This method depends upon the time necessary to reach the point where the starch-iodine color disappears. There are two objections to the Wohlgemuth method. First, the achromatic point occurs when practically all the substrate has been hydrolyzed, circa 80%. It is a principle of sound enzymology that the substrate should be present in such amount that the enzyme is always saturated. The second objection is that only one determination can be carried out at one time. This limits its usefulness where a large number of determinations are to be made.

Various modifications have been proposed to minimize or remove these objections. Instead of the achromatic point, it has been proposed that the hydrolysis proceed until a certain shade of color is reached. This has the advantage that the reaction is terminated before too high a percentage of the substrate is hydrolyzed, but it involves some subjective error in visual matching of the correct shade. Again, it limits the number of determinations to be made simultaneously to one.

The advent of the photometer and the spectrophotometer has removed these drawbacks. The incubation can be made for a definite period of time and percentage transmittance of the starch-iodine color then measured. This removes any subjective error. Also, the use of these instruments permits a large number of determinations to be made at the same time.

(c) Viscosimetric Techniques

Viscosimetric methods suffer from certain inherent weaknesses. These techniques depend upon the time necessary for a starch paste solution to lose a certain percentage of its initial viscosity, e.g. 20%. These methods are difficult to standardize and require careful preparation of the substrate if consistent results are to be obtained. In addition, only one determination can be made at one time, which again limits its usefulness.

(2). Method Adopted for this Study

The selection of a suitable method for the determination of amylase activity was therefore limited to either a saccharogenic or a dextrinogenic technique. Since it was proposed to investigate tissue amylases in addition to serum amylase, a dextrinogenic method was sought.

Of the many dextrinogenic methods proposed for the estimation of amylase activity, that of Huggins and Russell (17) seemed the simplest. Therefore, it was adopted with slight modifications for enzyme assay. It was found that the amylase activity of rat serum was quite high in comparison to human serum, and that a micro-modification could be used. The actual procedure followed is detailed hereunder.

Determination of Rat Serum Amylase Activity

(a) Reagents

Starch: a 1% solution of soluble starch (Eimer and Amend C.P.) was freshly prepared for each set of amylase determinations.

Buffer: 0.04 M phosphate buffer.

Stock Iodine Solution: 12.69 gm. iodine and 30 gm. KI in one litre H_2O. (0.1 N iodine).

Iodine Reagent: a 1:10 dilution of the stock iodine solution, containing 5% KF.

Serum: a 1:25 dilution of rat serum in 0.9% NaCl was usually employed.

(b) Procedure

Five millilitres of the freshly prepared starch solution and 5 ml. of the phosphate buffer were equilibrated in a test tube at 37° C. for five minutes. One millilitre of the diluted serum, also warmed to 37° C., was added; the tube was stoppered, and the contents were mixed by inversion. One millilitre of the mixture was transferred immediately to a 100 ml. volumetric flask which contained 4 ml. of the dilute iodine solution and the enzyme inhibitor, fluoride. At the end of one hour, 1 ml. of the digestion mixture was added to another 100 ml. volumetric flask containing 4 ml. of the iodine reagent. The contents of the flask were made up to 100 ml. with water and mixed. The percentage transmittance of the starch-iodine color was read in an Evelyn photometer

with filter 660 against a blank containing 4 ml. of the dilute iodine reagent which had been made up to 100 ml. The amount of starch present was read from the standard curve, (Figure 1), and the quantity of starch which had disappeared was then calculated.

The standard curve was prepared as follows: Samples of starch solution which increased in concentration by units of 0.5 mgm., and which covered the range from 0.5 mgm. to 5.0 mgm., were added to separate 100 ml. volumetric flasks, each containing 4 ml. of the dilute iodine reagent. They were diluted to 100 ml. with water, and read in the Evelyn photometer as above. A plot of the logarithm of the percentage transmittance against the concentration of starch resulted in a straight line passing through the origin.

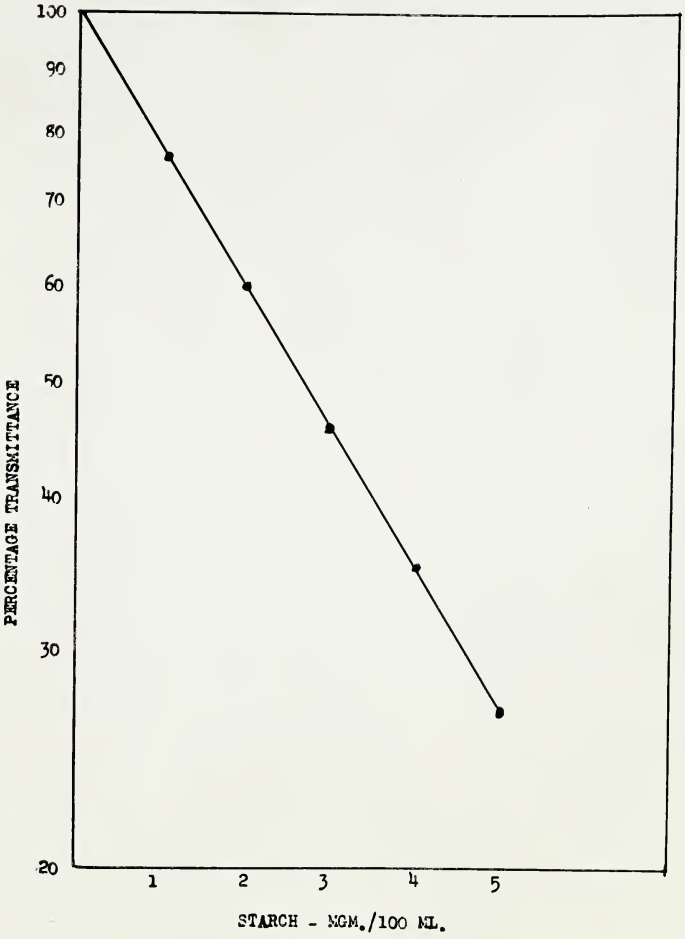

Figure 1. Standard Curve for Determination of Amylase Activity. Relationship between the logarithm of the percentage transmittance and the concentration of starch per 100 ml. of solution.

(c) Evaluation of the Method

(i) pH Optimum

It was first necessary to determine the optimum pH of rat serum amylase. A series of Sørensen 0.04 M phosphate buffers which varied in pH from 4.5 to 8.3 were prepared (24). These buffers increased in pH by 0.5 of a unit. Each buffer was checked on the Beckman pH meter before use. A series of determinations was carried out with these buffers and the amount of hydrolysis was calculated in each instance. This experiment was repeated on two further occasions and the results were averaged. A new series of buffers differing by 0.1 pH unit and covering the range from pH 6.8 to 7.3 was prepared and the same procedure repeated. During these experiments, pH determinations were made on samples at the beginning of the period of incubation, after 30 minutes of digestion, and at the termination of the incubation. In no instance did the drift in pH exceed 0.05 of a pH unit.

On the basis of these experiments, it was found that the pH optimum for rat serum amylase was pH 6.95. This pH was used thereafter for all amylase determinations. The pH activity curve is shown in Figure 2.

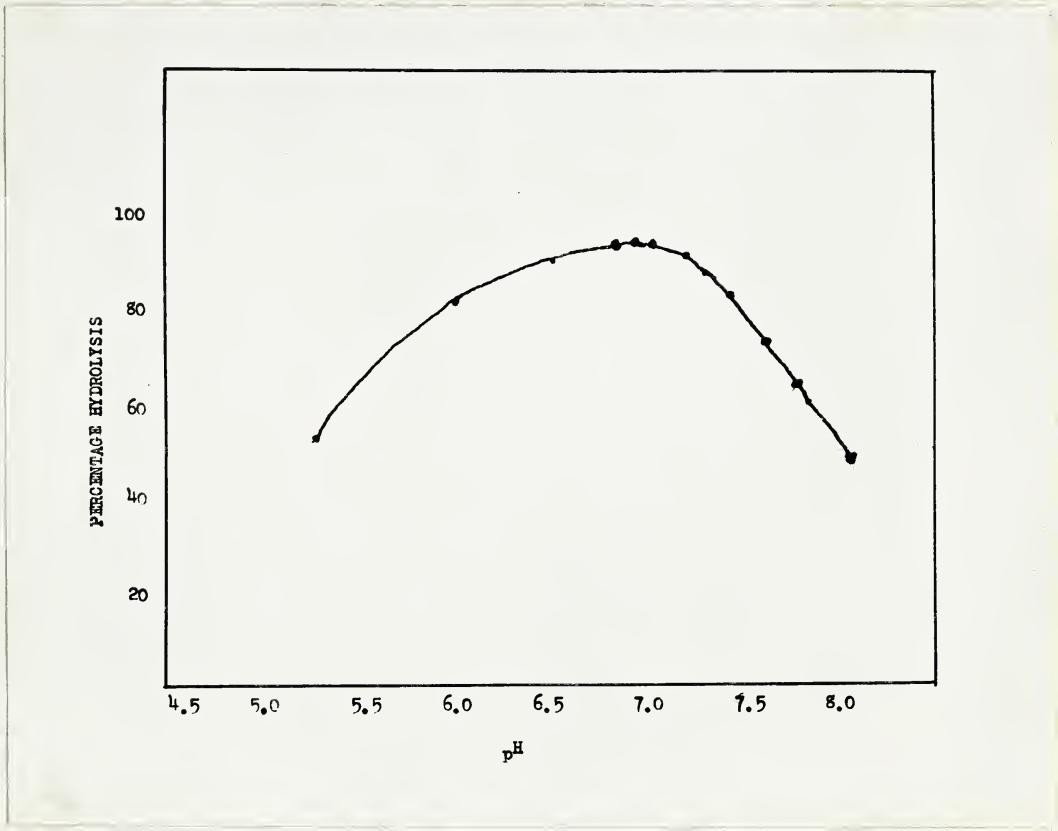

Figure 2. Relationship between the degree of hydrolysis and the pH with 0.04 M phosphate buffer for rat serum amylase.

(ii) Effect of Enzyme Concentration on Degree of Hydrolysis

A 1:50 dilution of rat serum was further diluted as follows:

	Enzyme	Water	Relative Dilution
1	1 ml.	5 ml.	0.5
2	2 ml.	4 ml.	1.0
3	3 ml.	3 ml.	1.5
4	4 ml.	2 ml.	2.0
5	5 ml.	1 ml.	2.5
6	6 ml.	-	3.0

Three millilitres of each of these solutions were incubated with the substrate and the buffer, and the number of grams of starch hydrolyzed was calculated. This procedure was repeated twice more and the results were averaged. The results of these tests are shown in Figure 3, as a graph in which the degree of hydrolysis is plotted against the enzyme concentration. A linear relationship was found to hold over the range of dilutions studied.

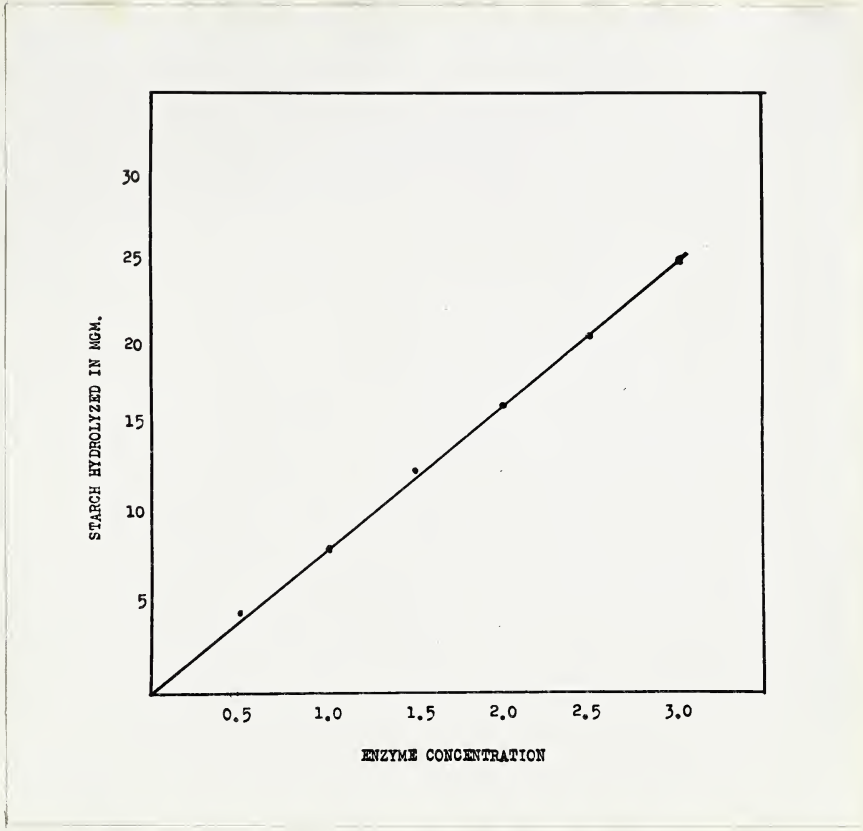

Figure 3. Influence of the enzyme concentration upon the degree of hydrolysis.

(iii) The Effect of Time of Incubation on the Degree of Hydrolysis

A 1:25 dilution of rat serum was incubated with buffer and substrate as above for varying periods of time. The length of the time ranged from 15 minutes to 120 minutes at 15 minute intervals. The amount of starch hydrolyzed in each digestion period was determined. This experiment was repeated twice more and the results were averaged. The degree of hydrolysis was plotted against the time of incubation (Figure 4).

The course of hydrolysis with time was linear, equal amounts of starch were hydrolyzed in equal amounts of time up to 120 minutes. A standard time of one hour was chosen for the length of the incubation, since it corresponded to the period of digestion selected by Huggins and Russell (17). This length of time also permitted the simultaneous assay of a large number of samples.

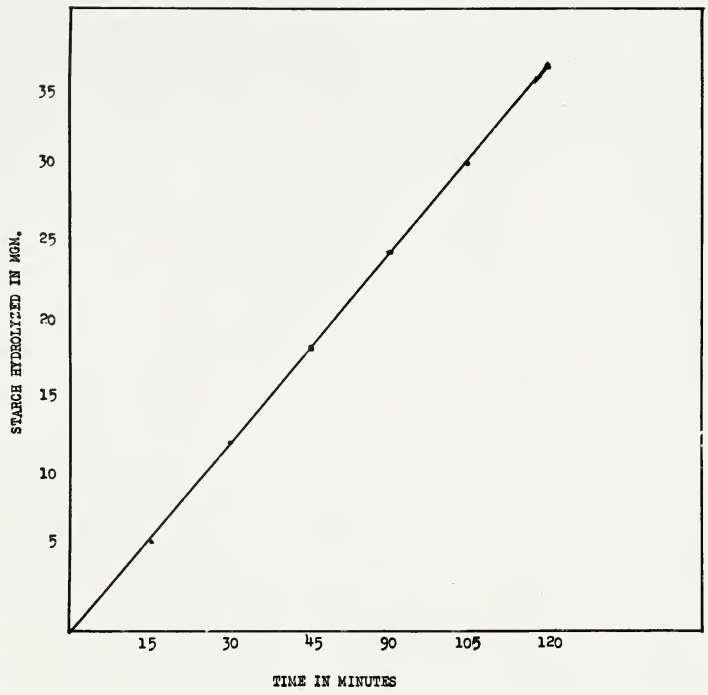

Figure 4. Time hydrolysis curve. The effect of the time of incubation upon the degree of hydrolysis.

(iv) Precision and Accuracy

Since the nature of the substrate prevented recovery experiments, a large number of replicates was assayed for amylase activity on several occasions. In each instance, these replicates agreed within 1% transmittance. As calculated in units of amylase activity, this would mean the method was accurate to plus or minus 2.5 units. This variation lies well within the range of the standard deviation and hence will not effect the validity of the results.

The photometer reading of the starch-iodine color was found to persist up to 24 hours at stable values. Huggins and Russell (17) also report that the starch-iodine color was stable for many hours both in the dark and the light.

(v) Unit of Amylase Activity

On the basis of the above investigations, the unit of amylase activity was defined as follows:

The unit of amylase activity is that amount of enzyme required to hydrolyze 1 gm. of starch in one hour at 37° C. and a pH of 6.95. Not more than 50% of the substrate should be hydrolyzed during the one hour digestion period.

Results were expressed as units of amylase activity per 100 ml. of serum or per 100 gm. of wet tissue.

B. Blood Sugar Determinations

In several instances, blood sugar determinations were made by the micro-method of Reinecke (19). This method depends upon the reduction of ferricyanide to ferrocyanide and the measurement of the "ferric ferrocyanide" complex photometrically.

C. Animals

Adult male rats of the Wistar strain were employed throughout these experiments unless otherwise specified. They were housed in individual cages and given food and water ad libitum. The standard laboratory diet of Purina Fox Checkers was fed in a powdered form to facilitate daily estimation of food consumption and to keep spillage at a minimum.

D. Collection of Serum

Blood was obtained by bleeding animals from the tail into 3 x 70 mm. glass tubes. The clotted blood was centrifuged, and the serum was removed with capillary pipettes. Then 0.1 ml. was diluted at once to 2.5 ml. with 0.9% NaCl. In the majority of cases, it was used at once for estimation of amylase activity, otherwise it was stored in the refrigerator at 5° C. A large volume of diluted blood serum was set aside in the cold for stability studies. The activity was measured daily for 10 days, then at weekly intervals for 100 days, then at monthly intervals for a total of 18 months. The enzyme preparation retained initial activity for the length of the investigations.

E. Statistical Analysis

Statistical methods were employed to test the significance of the experimental results. The Fisher *t* test was used for the comparison of significant differences between means. The correlation coefficient *r*, was used to appraise the relationship between two variables, e.g., serum amylase and food intake.

The criterion of significance was the probability value *P*. Results which exceeded the 5% level of *P* were regarded as significant, and those which exceeded the 1% level were regarded as highly significant. The methods employed were taken from L.P. Johnson's "An Introduction to Applied Biometrics". (100).

INVESTIGATIONS INTO RAT SERUM AMYLASE

1. Kinetics

A. pH Optimum

The pH activity curve of rat serum amylase showed a well-defined fairly broad optimum, with the greatest activity at pH 6.95 (cf. Figure 2). This is in agreement with some of the studies of human serum amylase (20, 21, 22) although Huggins and Russell (17) reported the optimum to be 7.4. Meyers, Free, and Rosinski (23) observed that the enzyme in human serum possessed a long broad range of optimal activity from 6.8 to 7.6. The pH optimum of an enzyme can often vary with the conditions of the incubation and this no doubt accounts for some of the discrepancies noted above. Huggins and Russell and Meyers et al used dextrinogenic methods for their enzyme determinations. Meyers et al did not employ a buffer and limited the time of incubation to 30 minutes.

B. Determination of the Michaelis Constant

The simplest case of an enzymic reaction may be given by the Mass Action Law:

$$K = \frac{(E)\ (S)}{(ES)} \qquad (1)$$

where K = dissociation constant, (E) = the concentration of the enzyme, (S) = the concentration of the substrate, and (ES) = the concentration of the enzyme-substrate complex. If the concentration (E) and (ES) is kept constant and the concentration of (S) varied, the initial velocity is given by the Michaelis-Menten equation:

$$v = \frac{Vmax\ (S)}{Km + (S)} \qquad (2)$$

where Vmax is a numerical constant representing the maximum velocity obtained when the enzyme E exists completely in the form (ES), i.e., Vmax = k E (total). Upon taking the reciprocal of both sides, equation 2 becomes linear:

$$\frac{1}{v} = \frac{Km}{Vmax \times S} + \frac{1}{Vmax} \qquad (3)$$

If equation 3 is multiplied through by S, it becomes:

$$\frac{S}{v} = \frac{Km}{Vmax} + \frac{S}{Vmax} \qquad (4)$$

In equation 4, when $\frac{S}{v}$ is plotted against S, the ordinate intercept is $\frac{Km}{Vmax}$ and the constant slope is $\frac{1}{Vmax}$.

Consequently, if various concentrations of substrate are incubated with a standard amount of enzyme and the results are plotted graphically, i.e. S/v against S, it will be possible to determine the ordinate intercept and the slope of the line. If these values are substituted in equation 4, it will be possible to determine the Michaelis Constant, Km.

The significance of the Michaelis Constant is that it represents the substrate concentration at which half the limiting velocity Vmax is attained. This constant is characteristic for each enzyme.

A series of incubations with different substrate concentrations was carried out in order to determine the Michaelis Constant for rat serum amylase. Five, 10, 15, 20, 25, and 30 mgm. of starch were incubated with 1 ml. of enzyme preparation under the usual conditions. The amount of substrate hydrolyzed was measured in each case and the following parameters were calculated:

S = substrate weight in mgm.

v = velocity of reaction (mgm. of starch hydrolyzed).

$\frac{1}{v}$ = reciprocal of velocity of reaction.

$\frac{S}{v}$ = substrate concentration X reaction velocity.

Data for this experiment are given in Table I.

When S/v was plotted against S, a straight line was produced with an ordinate intercept of 0.6 and a slope of 0.0803. Substitution of these values into equation 4 gave a Km value of 7.5 mgm./11 ml. (Figure 5).

Huggins and Russell (17) found the value for human serum amylase to be 7.14 mgm./10 ml. Hanes (26) obtained a Km value equivalent to 7.8 - 7.9 for the α amylase of sweet peas. Since the molecular weight of starch is not known, it is not possible to report the Km in the customary molar concentration.

A plot of substrate concentration against velocity (Figure 6) showed that the maximum velocity or saturation of enzyme with substrate was reached at about 30 mgm. per 11 ml. At 60 mgm. the substrate began to exert an inhibitory effect on the enzyme reaction. Consequently, a concentration of 50 mgm. was chosen for all subsequent studies. In this range any change in substrate concentration has a minimal effect on the velocity.

Table I

Data Used for the Determination of the Michaelis Constant

S	V	I/V	S/V
Weight of Substrate in mgm.	Starch Hydrolyzed in mgm.		
5	3.25	0.308	1.54
10	6.50	0.154	1.54
15	7.70	0.13	1.95
20	8.50	0.118	2.34
25	9.0	0.111	2.78
30	9.0	0.111	3.32

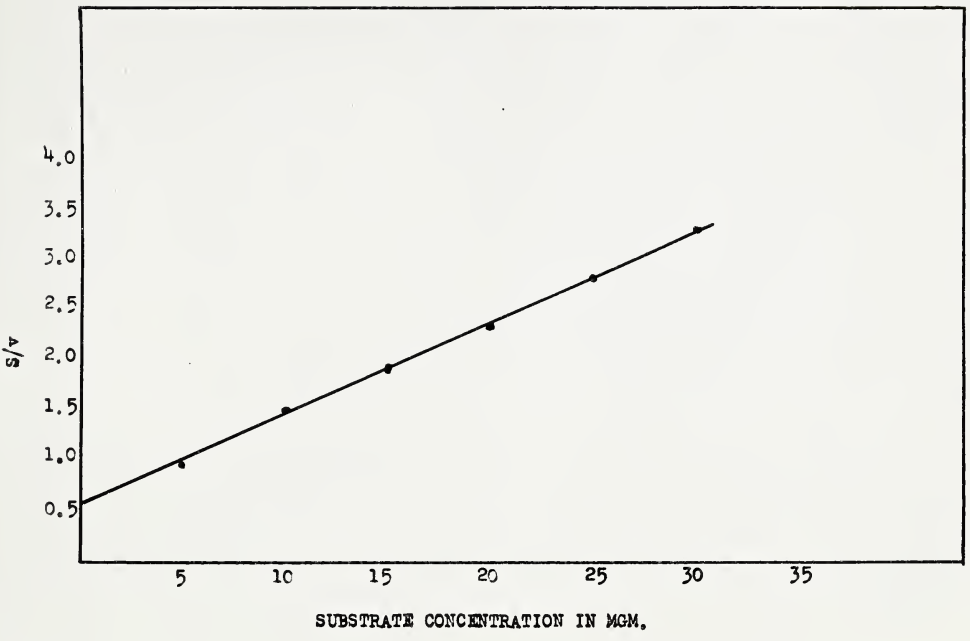

Figure 5. Determination of the Michaelis Constant. Relationship between S/v (substrate concentration times the reciprocal of the reaction velocity) and S, the substrate concentration. Ordinate intercept was 0.6 and the slope was 0.0803.

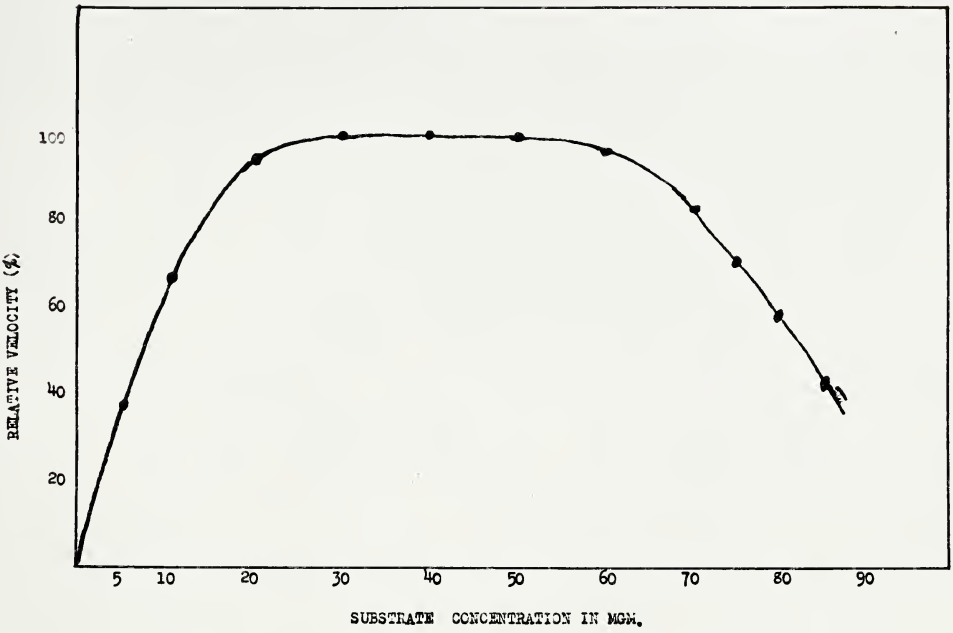

Figure 6. Effect of the substrate concentration upon the rate of hydrolysis.

II. Normal Values in Adult Rats

Normal adult amylase activity was determined on 100 adult male rats and 100 adult virgin female rats which had been maintained on the stock laboratory diet. Values for the males (plus or minus the standard error of the mean) were: 59.7 ± 1.6 units per 100 ml. of serum, and for the females: 49.0 ± 1.10 units per 100 ml. of serum. This difference between male and female levels is highly significant, ($t = 8.1$, $P < 0.01$). Tuba, Baker, and Cantor (27) noted a similar difference with rat serum alkaline phosphatase. Somogyi (11) states that in humans, differences in serum amylase levels due to sex were entirely negligible.

III. Diurnal Variations

Previous experiments by Tuba, Baker, and Cantor (27) have shown that a diurnal variation existed with rat serum alkaline phosphatase and was associated with food intake. Rat serum lipase (28) did not show a diurnal variation but remained relatively constant. The literature makes no reference to diurnal variations in serum amylase.

In these studies, a group of 10 adult male rats was used, and a pooled sample of blood, which consisted of 10 drops from each rat, was assayed for enzyme activity. Samples for amylase determination were collected at 8 a.m. and every three hours thereafter, for a total of 24 hours. The results of this experiment are shown in Table II. The experiment was repeated with a second group of 10 rats and essentially the same results were obtained. These also are shown in Table II.

The lowest level of amylase activity was found at 5 p.m. This is the fasting period in the rat and would correspond to the pre-breakfast level in humans. After the animals had taken food and digestion was in progress, there was a slow rise in amylase activity until a relatively steady level was attained at 2 a.m. This level remained more or less constant for 12 hours when the post-digestive value again became evident.

In a further experiment a group of 10 rats was bled individually at 9 a.m., 5 p.m., and at 8 p.m. to establish if the decline was significant. The following values were obtained:

9 a.m.	59.0 $\pm$ 2.4 (S.E.M.)
5 p.m.	51.2 $\pm$ 3.4
8 p.m.	58.1 $\pm$ 2.7

The difference between the 9 a.m. and the 5 p.m. level, and the difference

between the 5 p.m. and the 8 p.m. level were both highly significant ($P < 0.01$). This indicated that the serum amylase of rats was similar to their serum alkaline phosphatase in that it underwent a diurnal variation.

Table II

Diurnal Variation of Rat Serum Amylase

Time	Amylase activity in units/100 ml.	
	Group I	Group II
8 A.M.	58.4	59.6
11 A.M.	61.0	59.6
2 P.M.	62.0	56.8
5 P.M.	51.3	52.3
8 P.M.	59.6	60.8
11 P.M.	64.0	65.0
2 A.M.	65.0	66.8
5 A.M.	67.0	66.8
8 A.M.	65.0	65.0

IV. Effect of Age

A. Introduction

Somogyi (11) has noted the complete absence of amylolytic activity in the blood of newborn infants. He further reports that the enzyme usually first appears at the age of two months, that it can be measured quantitatively at three months, and reaches a relatively normal level in about one year. Tuba and Kovacs (29) also have found that the levels of serum amylase activity in infants were lower than those of adults. Mention of serum amylase levels in newborn animals cannot be found in the literature.

Investigations into serum amylase and age were carried out in two distinct stages. The first stage was concerned with suckling rats and it covered the period from birth until the time of weaning. The second phase of the investigation followed the enzyme level from weaning until maturity, i.e. 19 months.

B. Suckling Animals

At least 15 animals were sacrificed for each group since the amount of available blood was small. Pooled blood samples from four to five rats were used for each determination. The results of this investigation are given in Table III.

The outstanding feature of these results was that the serum amylase remained at a more or less constant level throughout the length of the nursing period. It was noticeable that the level of the enzyme was much lower than in the adult animals.

Table III

Serum Amylase Levels in Suckling Rats

Age in days	Serum amylase in units/100 ml.
1	23.5
2	23.5
3	17.9
6	20.0
7	22.3
8	24.7
10	22.0
12	23.5
17	22.3
20	25.0

Values shown are for the means of at least fifteen rats.

C. Serum Amylase Levels After Weaning

In this laboratory, rats are usually weaned at 21 days. A group of 10 male weanling rats was kept in separate cages and allowed food and water ad libitum. Routine serum amylase determinations were made, first at weekly intervals, then later at monthly intervals. These determinations were carried well into maturity (19 months). The results of this study are presented in Table IV.

The mean serum amylase level for this group at weaning was 44.2 units. This was considerably lower than the value reported for adult males (59.7 units). By the first week the enzyme level had risen to 54.0 units, and two weeks after weaning it reached adult levels. This value remained relatively constant until little over a year had elapsed.

At 380 days, these animals still possessed similar values for serum amylase, but at 411 days they had fallen to low levels. These lower levels persisted until the experiment was terminated. This decrease in serum amylase with advancing age is probably associated with decreased food intake. As will be noted later, the level of the enzyme in the blood is to a very large extent dependent upon the amount of food consumed. The food consumption of the older rats is considerably lower than that found in the younger more vigorous animals. Adult male rats up to one year in age consume from 20 to 24 gm. powdered checkers each day. The older rats (411 days or more) consumed only 15 gm. per day.

Table IV

Serum Amylase Levels in Weaned Rats

Age in days	Serum amylase in units/100 ml.
22	44.2
29	54.0
37	62.8
44	61.9
51	59.0
73	61.5
100	57.0
204	64.8
380	58.7
411	44.8
588	49.6

Values shown are for the means of ten rats.

V. Effect of Fasting

Tuba, Baker, and Cantor (27) noted that the food intake was able to influence the level of rat serum alkaline phosphatase. Rat serum tributyrinase, on the other hand, was not so readily affected (30, 31).

Eight male rats were maintained in individual cages on the usual dietary regime for a period of seven days. After this period of stabilization, the animals were fasted for 14 days, during which time water was always available. Blood was collected at the beginning and end of the fast and at the times indicated in Table V for determination of amylase values. Body weights were noted at each time of bleeding. Ad libitum feeding was resumed on the 15th day of the experiment and continued for 25 days, when the serum amylase and body weights were again determined.

The data in Table V indicate that serum amylase levels decreased during the fasting period to approximately 25% of the "normal" values by the fourteenth day. The decrease in activity by the third day of the fast was statistically significant ($t = 2.76$, $P < 0.05$). Amylase values and weights of the fasted rats were restored to normal after 25 days feeding. Three of the original eight animals failed to survive the prolonged fast and the results for these are not included in the data above. The serum amylase value for one of these shortly before death was 2.0 units per 100 ml.

Table V

Effect of Fasting on Rat Serum Amylase Levels

Fasting Period in days	Serum amylase Units/100 ml.	Body Weight in gm.
0	49.4 ± 2.7	301 ± 9.5
1	41.6 ± 2.5	272 ± 7.5
3	35.6 ± 3.8	249 ± 6.0
7	22.3 ± 2.7	219 ± 3.9
14	12.6 ± 3.9	172 ± 3.9
(food restored for 25 days)	48.3 ± 2.7	320 ± 10.1

Each value is the mean of five rats (plus or minus the standard error of the mean).

VI. Limited Food Consumption and Rat Serum Amylase

A. Normal Adult Male Rats

A further experiment was devised to determine the degree of correlation between daily food consumption and serum amylase values. Ten adult male rats were fed ground Purina fox checkers and water *ad libitum* for seven days. The average daily food intake was found to be 21.0 ± 1.3 gm. After the seventh day the quantity of the food placed before each animal was decreased by 5 gm. every three days thereafter. At the end of each period on each food level, values were obtained for body weights, and levels of serum amylase and blood glucose. The results of this investigation are given in Table VI.

The serum amylase values declined with decreasing food intake. The decreases in enzyme activity were paralleled by changes in body weight and blood sugar values. Statistical treatment of the data revealed: (1) a highly significant correlation between food consumption and serum amylase ($r = 0.77$, $t = 7.39$, $P < 0.01$), (2) a highly significant correlation between the levels of the enzyme and blood sugar ($r = 0.60$, $t = 4.56$, $P < 0.01$), and (3) a significant correlation between body weight and serum amylase ($r = 0.305$, $t = 2.52$, $P < 0.05$). It was not until the animals had been permitted to consume only 10 gm. of food daily for three days that the activity of serum amylase fell significantly below the *ad libitum* values ($t = 2.76$, $P < 0.05$). In this experiment n equals 40.

Table VI

Response of Normal Male Rats to Limited Food Consumption

Consumption gm./day	Serum Amylase units/100 ml.	Blood glucose mgm. %	Body weight gm.
21	55.2 ± 1.5	197 ± 12.0	345 ± 13.0
15	52.0	123	332
10	43.5	105	318
5	39.6 ± 1.2	77 ± 7.0	294 ± 10.0

Each value is the mean of ten rats (plus or minus the standard error of the mean at the beginning and end of the experiment).

Each food level was fed for three days before estimations were carried out.

B. Normal Adult Virgin Female Rats

A similar study of restricted food intake was made on normal adult virgin female rats. In this case, 4 gm. decrements were used and the animals were fed at each level for three days before serum amylase estimations were performed. These results are given in Table VII.

Maintenance on a subnormal daily food intake as low as 4 gm. did not produce significant lowering of the serum amylase values ($P > 0.05$). This would appear to indicate that in the female rat the correlation between the food intake and serum amylase is influenced by hormonal factors not present in the male.

C. Alloxan Diabetic Male Rats

If decreased food consumption resulted in decreased serum amylase, did the converse also apply? Would an increased food consumption lead to increased serum amylase levels? Cantor, Wight, and Tuba (32) reported that alloxan diabetic rats had increased serum alkaline phosphatase activity, while Tuba and Hoare (30) reported similar findings for rat serum tributyrinase. These increased levels were subsequently found to be due to the enhanced food consumption (31, 33).

Table VII

Response of Virgin Female Rats to Limited Food Consumption

Consumption gm./day	Serum amylase units/100 ml.	Body Weight gm.
16 (ad libitum)	41.2 ± 1.20	189 ± 7.18
12	42.2 ± 1.22	186 ± 5.93
8	35.5 ± 2.77	175 ± 5.71
4	36.4 ± 2.00	162 ± 5.47

Each value is the mean of six rats (plus or minus the standard error of the mean).

Each level was fed for three days before estimations were carried out.

A group of 10 male adult rats, with well manifested alloxan diabetes, was placed on a similar experimental protocol with respect to limited food intake, to that already reported for the normal males. Results of this experiment are to be found in Table VIII. Alloxan diabetes was achieved by the subcutaneous injection of alloxan (200 mgm./kgm. body weight) into rats which had been fasted for 48 hours. Blood glucose levels were followed at weekly intervals until a diagnosis of alloxan diabetes could be established. The usual clinical symptoms of polydipsia, polyuria, and polyphagia were also present. Reference to glucose levels in Table VIII will show that these animals were severely diabetic.

The ad libitum food consumption in Table VIII was significantly higher than the levels in Table VI for normal males. In spite of this, serum amylase values are not significantly different in the two experiments in the cases where the animals were permitted to eat without restraint. There exists no correlation between food consumption and enzyme level in the diabetic animals. In fact, serum amylase activity even at the 5 gm. level of daily feeding is still statistically unchanged from the initial values.

This abnormality in the diabetic animals is emphasized by the fact that at the 5 gm. level of consumption the normal animals manifest a highly significant decrease in serum amylase activity compared with the ad libitum feeding ($t = 4.57$, $P < 0.01$). So while it cannot be said that an abnormal elevation exists in alloxan diabetic animals on an ad libitum diet, there was obviously a departure from the normal response in decreasing daily food intake. This doubtless is associated with the altered metabolic activities of the diabetic animals. Hecht (34) and

Table VIII

Response of Alloxan Diabetic Male Rats to Limited Food Consumption

Consumption gm./day	Serum amylase units/100 ml.	Blood glucose mgm. %	Body weight gm.
27.0 ± 1.1	51.7 ± 2.8	434 ± 3.0	299 ± 9.8
25.0	55.6	353	296
20	58.0	345	291
15	53.6	171	285
10	50.2	132	268
5	52.6 ± 2.6	141 ± 6.6	248 ± 9.1

Each value is the mean of ten rats (plus or minus the standard error of the mean at beginning and end of experiment).

Each level was fed for three days before estimations were carried out.

Lukens (34) also noted that serum amylase levels in alloxan diabetic rats did not vary materially from normal animals.

VII. Effect of Sex Hormones on Normal Rats

A. Introduction

It was observed that a highly significant difference existed between serum amylase levels in the normal male and female rat. It seemed probable that the lower enzyme activity in the female rat might be associated with a lower daily intake of food, as in the case of rat serum alkaline phosphatase (27). The sexual variation of serum amylase levels in the normal rat might therefore be emphasized following injection of male and female sex hormones.

B. Normal Male Adult Rats

For the first part of this experiment, 24 adult males were divided into four groups of six. They were fed ad libitum on the usual diet in individual cages for seven days and then they were bled for serum amylase estimation. Group I was left as a control group and received no injections. Group II was injected daily with 0.2 ml. of oil of sesame. Group III received daily injections of 1.0 mgm. testosterone propionate in 0.2 ml. oil of sesame. Group IV was injected daily with 0.1 mgm. oestradiol dipropionate in 0.2 ml. oil of sesame. The injections were made subcutaneously and continued for a period of seven days. At the end of the seven day period the animals were bled again for serum amylase determinations. The results are presented in Table IX.

A slight fall of about 10% was found in the enzyme levels of the animals in Group III which were injected with testosterone, and this was approximately the same as the decrease which occurred in the rats injected with oil of sesame (Group II). This may be attributed to slightly decreased food intake in these two groups which is more marked than in the control animals of Group I. There was no significant weight loss in spite of decreased food consumption, so it may be assumed that the oil of sesame compensated to a large extent for the caloric loss involved. The injection of oestradiol in Group IV produced the most striking results. Enzyme activity fell by 17% while food intake decreased by 60%, and there was a weight loss of 35 gm. in seven days. A statistical analysis of Group IV indicated that the effect of the injected female sex hormone produced decreases in serum amylase activity

Table IX

Effect of Sex Hormone Injections on Normal Male Adult Rats

Group	Substance injected	Food Consumption gm./day.	Body Weight gm.	Serum amylase units/100 ml.
I	-	24 ± 1.5 21 ± 1.5	381 ± 7.6 394 ± 7.9	66.5 ± 2.0 63.1 ± 1.8
II	Oil of sesame	24 ± 1.0 17 ± 0.4	353 ± 8.7 351 ± 10.0	61.2 ± 1.4 56.5 ± 1.4
III	Testosterone propionate	24 ± 2.2 16 ± 0.8	365 ± 14.0 360 ± 13.4	62.6 ± 2.9 56.8 ± 2.5
IV	Oestradiol dipropionate	23 ± 1.20 9 ± 0.73	355 ± 13.8 319 ± 13.0	57.2 ± 1.6 47.3 ± 2.0

Each value shown is the mean of six animals (plus or minus standard error of the mean).

Upper figures of each pair represent value at commencement of hormone treatment, while lower figures represent values after seven days on the experiment.

which were highly significant ($t = 5.0$, $P < 0.01$), whereas the differences in the other groups were not significant. The correlation between food intake and serum amylase in Group IV was highly significant ($r = 0.838$, $t = 4.81$, $P < 0.01$) as was the case with the data of Table VI.

C. Normal Adult Virgin Female Rats

The second part of this experiment was the same as the first except that female rats were used instead of males. The results are reported in Table X. There were no significant changes in values of food consumption, body weight, or serum amylase for the first three groups. The amount of food eaten by the control female rat was significantly lower than for male controls (Group I in Tables IX and X, $t = 3.60$, $P < 0.01$). Oestradiol reduced food consumption profoundly and to about the same values as in Table IX. There was once more an accompanying drop in weight although not to the same degree as with the males. There were no significant alterations in enzyme levels produced by oestradiol although a comparison of the four groups indicates a lower trend in Group IV. Weights in Group IV returned to normal after the injections were discontinued. Food consumption and enzyme levels were abnormally high seven days after cessation of oestradiol treatment, but after 14 days they had once more returned to values characteristic of female rats. The lack of correlation between the serum amylase and the food consumption contrasts markedly with the results for male rats, but it will be recalled from Table VII that the serum amylase of female rats is much more refractory to diminished food intake than that of male rats in Table VI.

Table X

Effect of Sex Hormones on Normal Virgin Female Adult Rats

Group	Substance injected	Food Consumption gm./day	Body Weight gm.	Serum amylase units/100 ml.
I		17 ± 1.4	198 ± 3.3	49.2 ± 1.0
		15 ± 1.5	202 ± 6.0	55.0 ± 4.3
II	Oil of sesame	17 ± 0.6	197 ± 1.9	49.9 ± 2.8
		15 ± 0.7	205 ± 1.6	53.8 ± 2.6
III	Testosterone propionate	16 ± 1.1	195 ± 3.5	45.4 ± 0.8
		15 ± 0.7	212 ± 1.6	52.7 ± 3.6
IV	Oestradiol dipropionate	16 ± 0.6	201 ± 2.5	45.0 ± 0.5
		10 ± 0.3	188 ± 0.8	43.4 ± 0.5
		20 ± 0.8 (1)	198 ± 2.9	61.2 ± 2.7
		15 ± 1.1 (2)	208 ± 1.2	54.2 ± 2.4

Values shown are the means of six animals (plus or minus the standard error of the mean).

Upper figures of each pair represent values at commencement of hormone treatment, while lower figures represent values after seven days on the experiment.

(1) Values seven days after cessation of hormone treatment.

(2) Values fourteen days after cessation of hormone treatment.

These results were in all probability modified by the sex hormones present in the intact animals of both sexes, so it was decided to repeat these experiments with castrate male and female rats.

VIII. Gonadectomy

A. Orchidectomy

1. Effect of Castration

Thirty adult male rats were fed the standard laboratory diet for a period of seven days, then serum amylase activities were obtained in all the animals, following which 24 of the group were castrated. Activity of the enzyme was determined one week later for the castrated rats and also for the control group of six adult rats. These estimations were repeated at weekly intervals until four weeks after the operations had been performed. No enzyme values were obtained before seven days had elapsed, because the surgical trauma produced a post-operative anorexia, which sometimes persisted for as long as five days, and which could have induced the sub-normal enzyme levels associated with food consumption. The results of this experiment are given in Table XI.

The data in Table XI indicated that one week after the operation enzyme levels had decreased in the castrated group, and the difference between the control group and the orchidectomized animals was highly significant ($t = 5.0$, $P < 0.01$). This significant difference disappeared by the 14th day following the operations and values returned to normal, hence only a transient effect on serum amylase resulted from orchidectomy.

Table XI

Influence of Castration on Normal Male Rats

Time days	Serum amylase units/100 ml. Control	Serum amylase units/100 ml. Castrate	Food consumption gm./day Control	Food consumption gm./day Castrate	Body weight gm. Control	Body weight gm. Castrate
Pre-operative	62.5 ± 1.4	62.8 ± 2.3	22	22	326 ± 8.7	327 ± 7.2
Post-operative						
7	58.1	50.6	23	21	340	316
14	60.5	56.8	21	21	348	325
21	58.2	55.9	20	21	365	342
28	59.4 ± 2.8	60.3 ± 2.1	20	20	374 ± 8.7	363 ± 9.1

Each value is the mean of the group (plus or minus the standard error of the mean at the beginning and end of the experiment).

2. Effect of a Sham Operation

A similar, statistically significant, but transient fall in serum amylase values followed ovariectomy (see below). It was felt that the results in both instances might be associated with surgical trauma and the subnormal food intake of the first few post-operative days. Consequently, a group of six normal adult rats were subjected to incisions, about 4 cm. in length, right through the abdominal wall. These incisions were immediately sewn up. Amylase levels, food consumption and body weights were observed for two weeks in the group subjected to the sham operation and in a control group of six normal adult male rats. These results are presented in Table XII.

The amylase values of the control group did not vary significantly during the experiment. However, the sham operation produced transient but highly significant decreases in the serum enzyme ($t = 7.00$, $P < 0.01$) and these were paralleled by decreased food intake and by weight losses. By the end of the second post-operative week, the activity of the serum amylase in the experimental group had returned to values which were no longer statistically different from normal. This is evidence that surgical trauma and associated anorexia may produce a statistically significant but transient diminution of rat serum amylase activity.

Table XII

Response of Normal Male Rats to a Sham Operation

Time Days	Serum amylase units/100 ml.		Mean daily food consumption gm./day/rat over 7 days		Body weight gm.	
	Control	Experimental	Control	Experimental	Control	Experimental
Pre-operative	54.0 ± 2.5	53.1 ± 2.8	19	19	379 ± 11.8	364 ± 10.6
Post-operative						
7	59.4 ± 2.2	36.0 ± 2.0	21	12	384 ± 10.3	319 ± 11.3
14	58.6 ± 2.4	50.4 ± 3.1	22	20	394 ± 10.6	342 ± 8.4

Each value is the mean of the group (plus or minus the standard error of the mean).

3. Effect of Sex Hormones on Castrates

After the serum amylase levels of the castrated male rats had returned to normal values, the animals were divided into four groups of six rats each. One group received daily injections of 1.0 mgm. testosterone propionate, another group received daily injections of 0.1 mgm. oestradiol dipropionate. These hormones were contained in 0.2 ml. of oil of sesame, and this amount of the oil was daily injected into each rat in a third group of castrates. Injections were made subcutaneously for a period of seven days. The fourth group of castrates received no injections. A group of normal animals was used as a further control group for the experiment. Amylase determinations were made immediately preceding and following the hormone treatment. The results are recorded in Table XIII.

The data in Table XIII revealed that testosterone was without effect on the serum amylase of the castrated male rat, and that body weight remained unchanged in spite of a slight diminution of food intake. The effect of the oil of sesame injections was not significant. Oestradiol dipropionate produced a loss in weight, a pronounced decrease in food consumption, and a significant decrease in serum amylase levels ($t = 5.8$, $P < 0.01$). The data for the normal and the castrate control group showed significant variation during the experiment.

These findings are in agreement with the previous similar experiment with intact males. It appears that the female hormone can profoundly effect the food intake and serum amylase levels of either normal or castrated male rats, and that the testes do not in any way modify this effect.

Table XIII

Effect of Sex Hormones on Castrate Male Rats

	Group I Normal control	Group II Castrate control	Group III Testosterone propionate	Group IV Oestradiol dipropionate	Group V Oil of sesame
Serum Amylase Units/100 ml.	58.2 ± 2.8 61.4 ± 2.2	53.9 ± 1.8 56.7 ± 2.3	58.5 ± 1.5 62.0 ± 2.4	57.3 ± 2.1 50.3 ± 1.8	58.4 ± 2.0 54.2 ± 2.1
Food consumption gm./day	20 22	22 20	22 18	20 12	20 19
Body weight gm.	365 ± 8.7 366 ± 8.4	332 ± 7.9 334 ± 7.5	349 ± 8.0 354 ± 7.6	342 ± 6.8 315 ± 6.9	345 ± 8.0 338 ± 9.3

Each value is the mean of six rats (plus or minus the standard error of the mean where statistically necessary).

Upper figures in each pair represent values at commencement of hormone treatment, while lower figures in each pair represent values after seven days of hormone treatment.

B. Oophorectomy

1. Effect of Castration

Thirty adult female rats were maintained in individual cages on the usual laboratory diet for a stabilizing period of seven days. At the end of this period serum amylase levels were determined, and 24 of the animals ovariectomized. Enzyme levels were determined in a group of six normal rats and in the castrated animals at weekly intervals for a period of three weeks. The results of this experiment are given in Table XIV.

One week following ovariectomy, the mean serum amylase values had fallen significantly from the pre-operative levels for this group ($t = 5.6$, $P < 0.01$). By the end of the second post-operative week, levels of the enzyme were no longer significantly different from the pre-operative values, and food consumption and weight gains were somewhat greater than the normal control female. The third week showed the same trend as the second week and the experiment was then discontinued.

It would appear once more, as in the case of the male castrate rats, that the removal of the gonads produced only temporary diminution of serum amylase activity. This decrease in the levels of the enzyme may once again, as in the case of the male castrates, be accounted for on the basis of surgical trauma and decreased food ingestion during the immediate post-operative periods. It is obvious from Tables V, VI, VII, and XII, that the response of serum amylase to lowered food intake is slow and this accounts for the time lag between the time that the animals are eating normally and the return of the enzyme level to normal values.

Table XIV

Effect of Castration on the Normal Virgin Female Rat

Time in days	Serum amylase units/100 ml. Normal	Serum amylase units/100 ml. Castrate	Food consumption gm./day Normal	Food consumption gm./day Castrate	Body weight gm. Normal	Body weight gm. Castrate
Pre-operative	46.0 ± 2.4	49.3 ± 2.8	14	15	217 ± 6.0	220 ± 5.4
Post-operative						
7	47.9	40.7	15	14	219	230
14	45.7	48.3	16	18	225	251
21	47.4 ± 2.0	48.2 ± 2.2	15	18	233 ± 6.4	264 ± 7.4

Each value is for the mean of the group (plus or minus the standard error of the mean at beginning and end of experiment).

2. Effect of Sex Hormones on Castrate Female Rats

The effect of injections of testosterone propionate and oestradiol dipropionate were tested with the castrated females used in the preceding experiment. This study was identical with that carried out with the castrated male rats. The results will be found in Table XV.

The serum amylase values in Table XV indicate that there was no effect on the levels of the enzyme following injections of testosterone propionate, oestradiol dipropionate, or oil of sesame. These results are of especial interest with respect to those animals receiving oestradiol dipropionate. Despite the profound decrease in food consumption, there was no concomitant fall in the levels of the serum amylase. This recalls the results of the experiments given in Tables VII and VIII. The serum amylase in female rats appears to be more resistant to decreased food consumption than does that of the male.

Table XV

Response of Castrated Female Rats to Sex Hormones

	Group I Normal control	Group II Castrate control	Group III Testosterone Propionate	Group IV Oestradiol Dipropionate	Group V Oil of sesame
Serum amylase units/100 ml.	47.9 ± 1.4 45.7 ± 1.7	46.7 ± 2.6 49.3 ± 2.4	50.7 ± 2.5 51.7 ± 2.8	48.0 ± 2.7 45.8 ± 2.1 62.0 ± 3.6 (1) 50.5 ± 3.2 (2)	47.2 ± 2.2 48.7 ± 2.4
Food consumption gm./day	16 15	17 16	18 17	18 10 16 (1) 18 (2)	18 15
Body weight gm.	225 ± 4.7 225 ± 6.0	249 ± 8.1 245 ± 8.5	250 ± 6.0 257 ± 5.5	251 ± 7.8 231 ± 8.4 229 ± 9.0 (1) 231 ± 9.1 (2)	253 ± 4.5 244 ± 4.2

Each value is the mean of six rats (plus or minus the standard error of the mean where statistically necessary).
Uppermost figures in each set represent values at commencement of treatment.
Lowermost figures represent values after seven days of hormone treatment.
(1) - Values seven days after hormone treatment stopped.
(2) - Values fourteen days after hormone treatment stopped.

IX. Hepatectomy

Early investigation established that the liver was able to break down glycogen to glucose. The enzyme responsible for this transformation was referred to as an amylase or a glycogenase (11). The experiments of Cori and his colleagues (101) showed that the route of glycogenolysis was entirely different and involved the mediation of phosphorolysis rather than a hydrolytic mechanism. The vast size of the liver, its rich blood supply, and its importance in carbohydrate metabolism, led many of these early workers to maintain that the liver was a source of serum amylase (11). Further evidence was also available. Hepatocellular damage by liver poisons such as CCl_4 and $CHCl_3$ caused extensive decrease in serum amylase levels in cats and dogs (36, 37). Also, Gray, Probstein, and Heifetz (38) found that activity of the enzyme was significantly low in most cases of liver disease in humans. Glock (39) reported the presence of an amylase in the perfused blood-free liver of a number of animals including the rat, although she was unable to find any amyloclastic activity in human serum. Critical examination of her methods show that she was measuring phosphorolysis as well as amylolysis. It is stated by Myrbäck and Neumüller (40) that blood-free liver powder possesses some amylase activity.

In consideration of the above factors, it was felt that the liver should be investigated as a possible source of serum amylase activity in the rat. Two routes of investigation were available: (1) the administration of liver poisons, and (2) removal of part of the liver tissue. The first technique was not employed since CCl_4 injection is accompanied by anorexia (which would obscure the results), and $CHCl_3$

is highly lethal to rats (102). Accordingly, it was decided to proceed by the second alternative, that of hepatectomy. Certain points distinguish these two techniques. Liver has great functional reserve power against anatomical removal of the tissue, but liver poisons readily depress functional activity. However, it was felt that if a large portion of the tissue was excised, any contribution it might make to the level of serum amylase would be reflected in altered enzyme values.

Partial hepatectomies, (involving the removal of the right and the median lobes of the liver) were performed on six normal male rats. Serum amylase values were determined immediately prior to the operation and they were followed post-operatively at weekly intervals for four weeks. Six normal rats of approximately the same age served as controls. These results are recorded in Table XVI.

The overall effect of partial hepatectomy appeared to be negligible. The usual transient decline in serum amylase levels was noted on the eighth day. This highly significant decrease, ($t = 3.76$, $P < 0.01$) had disappeared by the fourteenth day and normal values were found on the 21st and 28th days after the hepatectomies. Food consumption was normal by the end of the fourth day and did not deviate appreciably from the controls. It should be noted that the hepatectomized animals did not lose weight, as was the case with the rats which had been subjected to gonadectomy or to the sham operation.

On the basis of this experiment it would appear that the liver contributes very little to the maintenance of serum amylase levels in the rat. In a group of six adult males, the serum contained 64.7 ± 6.9 units of amylase activity per 100 ml., while the liver con-

Table XVI

Partial Hepatectomy and Serum Amylase Activity

Time in days	Serum amylase Units/100 ml. Control	Serum amylase Units/100 ml. Hepatectomized	Food consumption gm./day Control	Food consumption gm./day Hepatectomized	Body weight gm. Control	Body weight gm. Hepatectomized
Pre-operative	60.9 ± 2.4	58.5 ± 1.6	20	21	212 ± 6.5	219 ± 8.1
Post-operative						
8	59.3	51.4	20	20	239	237
14	62.9	60.5	20	21	269	264
21	63.0	63.1	21	21	284	287
28	62.4 ± 2.0	62.1 ± 2.5	24	23	303 ± 7.4	314 ± 8.4

Each value is for the mean of six rats (plus the standard error of the mean at the beginning and the end of the experiment).

tained 13.5 $\pm$ 2.8 units/100 gm. wet tissue. Even if one assumed that the liver was entirely blood free, (the animals were killed by decapitation and some residual blood undoubtedly remained), it seemed improbable that the contribution to the serum amylase by the liver would be significant. These findings are in agreement with those reported by Somogyi (11). Flock and her co-workers (41, 42) have found normal plasma amylase levels in totally and partially hepatectomized dogs. They note further that normal dogs have plasma enzyme activity of a magnitude similar to that observed in dogs with cirrhosis achieved by repeated administration of CCl_4. It was also reported by these investigators that the amylase activity of plasma drawn simultaneously from the femoral, hepatic, and portal veins of dogs was the same.

X. Pancreatectomy

A. Experimental

In this experiment, 11 partially pancreatectomized adult male rats were used as well as six normal males as controls. Because of the very diffuse distribution of pancreatic tissue, it was not possible to effect complete removal, but it is estimated that approximately 80 - 90% was extirpated. Fatalities following this operation were quite high, so it took several days to obtain sufficient pancreatectomized rats for statistical study. Hence the post-operative time shown in Table XVII represents the average for the group in each instance. The effect of this is most apparent with the data for the seventh post-operative day, when some of the animals were still suffering from surgical trauma and others had recovered to a large extent. Results of this experiment are reported in Table XVII.

B. Results

The first post-operative data in Table XVII show serum amylase levels which were abnormally low, accompanied by a low food intake. The slight weight gain for the group was due to the animals which had been operated on earliest and were manifesting recovery from the trauma. Food consumption was normal by the fourteenth day and then continued at levels almost 50% above normal for the duration of the experiment, or, more simply, for almost a year. During the experimental period there continued to be a steady weight gain in the depancreatized rats, which was however, appreciably less than in the case of the controls. Throughout the entire investigation the serum amylase levels

Table XVII

Influence of Pancreatectomy on Serum Amylase Levels

Time in days	Serum amylase units/100 ml. Control	Serum amylase units/100 ml. Depancreatized	Food consumption gm./day Control	Food consumption gm./day Depancreatized	Body weight gm. Control	Body weight gm. Depancreatized	Blood sugar mgm.% Control	Blood sugar mgm.% Depancreatized
Pre-operative	60.9 ± 3.4	60.0 ± 2.2	20	21	212 ± 6.5	230 ± 7.3	135 ± 7.6	143 ± 6.2
Post-operative								
7 (about)	60.0	46.1	21	13	238	235	106	174
14	61.3	48.0	20	23	269	263	127	184
42	59.0	48.0	20	33	303	282	115	219
332	63.5 ± 2.6	46.8 ± 1.8	20	32	492 ± 32	390 ± 32	118 ± 12.5	257 ± 22.0

Each value is for the mean of the group (plus or minus the standard error of the mean, where indicated).

NOTE: The above data were determined at weekly intervals for six weeks, then at monthly intervals.

Because there were no significant variations from the above trends, only a few values are included in the table.

in the depancreatized group were consistently and significantly below normal (in all cases $P < 0.01$), and the animals manifested a pronounced hyperglycemia. The urinary output for one animal was 160 ml./day as compared with the normal daily excretion of 7 - 10 ml. It was quite evident that pancreatic diabetes had been produced in the 11 animals of the operated group and that this decline was accompanied by a permanent and highly significant decrease in the levels of serum amylase.

C. Discussion

Similar findings have also been reported by other workers. Friedman and Thompson (43), Markowitz and Hough (44), and Zucker, Newburger, and Berg (45) found that complete pancreatectomy in the dog was followed by a sharp fall in serum amylase level. In some instances, after a lapse of time, the levels tended to return to normal although in other instances low values persisted throughout the length of the experiment. Roe and Goldstein (46) reported that pancreatectomy caused a post-operative decrease in serum amylase of cats with a mixed return trend to normal levels. Reid, Quigley, and Myers (47) found that the blood amylase of depancreatized dogs decreased the first few days after the operation but returned to the pre-operative level or even higher if the resultant diabetes was controlled with insulin. Cessation of insulin treatment resulted in a decreased amylase level which was restored to normal following resumption of insulin treatment. These workers also reported a decreased serum amylase level after insulin injection into intact dogs.

On the other hand, Roe, Smith, and Treadwell (48) found that levels of serum amylase in rats were not altered significantly by complete pancreatectomy up to 30 days following the operation. These differences in results were undoubtedly due to variations in experimental technique. Two factors which were probably involved were the diurnal variation and the diet. The protocol of Roe et al (48) was such that the collection of blood samples took place at the lowest levels of the serum amylase, whereas the procedure followed here coincided with the highest level of the diurnal variation. The diurnal variation is a digestive phenomenon associated with the activity of the pancreas and therefore would only be noticeable in intact animals. The "fasting" amylase level in the blood is not appreciably higher than the values found in the pancreatectomized animals; certainly no significant difference can be established. (Cf page 33 and Table XVIII.)

The animals used in the experiment of Roe et al (48) were also maintained on a synthetic "low residue diet" with the following composition: 30% casein, 25% lard, 14% starch, 14% sucrose, 5% salt mixture, 10% brewers yeast, 2% cod liver oil. It is not possible to predict the effect of such a diet on serum amylase levels, but it should be noted that alterations in the composition of a diet can profoundly effect levels of the serum amylase in the intact rat. The influence of diet on serum amylase levels is discussed more fully in a following section.

The response of serum amylase to pancreatectomy contrasts very markedly to the response to alloxan diabetes (cf. Table VIII). The alloxan diabetic rats were found to possess normal levels of the enzyme which did not drop with diminished food intake as was the case with the normal animals (Table VI). In the alloxan diabetic rat the acinar tissue, responsible for the elaboration of pancreatic amylase, is intact, while the absence of this tissue must be the reason for the decreased serum amylases noted above in the depancreatized animals. One would be justified in assuming that a very significant fraction of the serum amylase is pancreatic in origin since the decreases in the enzyme level noted in Table XVII were accompanied by a loss of at least 80% of the acinar tissue, and these decreases are maintained in spite of a persistent polyphagia. On the other hand, the fact that so much serum amylase activity remains following pancreatectomy may be taken as presumptive evidence that tissues other than the pancreas contribute to the maintenance of normal levels of the enzyme.

XI. Hypophysectomy

It was shown by Li, Kolman, and Evans (57) that serum alkaline phosphatase levels in growing rats could be decreased by removal of the hypophysis, and restored to normal following administration of the anterior pituitary growth hormone to the hypophysectomized animals. It has been established that there is a highly significant correlation between levels of serum alkaline phosphatase and daily food consumption in normal and alloxan diabetic animals (32, 33), and that a similar situation exists between food intake and serum amylase in normal rats (Table VI). Inasmuch as the growth hormone elaborated by the anterior pituitary is intimately related to the metabolic processes which are dependent upon the level of the food intake, it seemed possible that the hypophysis might exert some influence on the levels of serum amylase.

Hypophysectomies were performed on a group of 10 male rats, 45 days old. Two weeks after the operation a group of five of these animals received daily injections of anterior pituitary growth hormone. The daily dosage was 0.2 mcgm. of the crystalline pituitary growth hormone in 0.2 ml. physiological saline. At the same time, 10 male rats of about 60 days old were divided into two groups of five each; one served as a normal control, and the other group received injections of the growth hormone. At the end of the period of injection, all the animals were killed by decapitation, in order to obtain enough serum for amylase determinations from some of the smaller animals. The results of this experiment are to be found in Table XVIII.

Table XVIII

Effect of Anterior Pituitary Growth Hormone on Hypophysectomized and Normal Young Male Rats

	Normal animals not treated with Growth Hormone	Normal animals treated with Growth Hormone	Hypophysectomized untreated animals	Hypophysectomized treated with Growth Hormone
Serum amylase units/100 ml.	65.4 ± 4.3	61.3 ± 3.2	42.5 ± 2.4	59.4 ± 2.6
Food consumption gm./day	21	21	9	15
Body weight in gm.	271 ± 8.0	273 ± 7.3	153 ± 6.2	215 ± 7.1

Each value is for the mean of the group (plus or minus the standard error of the mean).

The data in Table XVIII show that the serum amylase values for hypophysectomized rats, not treated with growth hormone, were significantly lower than the normal untreated animals ($t = 7.4$, $P < 0.01$). The hypophysectomized group treated with growth hormone manifested activity of the serum enzyme which was not significantly different from the normal untreated group. Normal animals which also received treatment with the hormone did not show any significant alteration in serum amylase values from those for the normal control group which were not injected.

Levels of the enzyme in the experimental animals paralleled the food consumption. Correlation between food consumption and values of serum amylase in Table XVIII was highly significant ($r = 0.86$, $P < 0.01$). The effects of hypophysectomy and injections into hypophysectomized animals of the pituitary growth hormone were therefore almost entirely associated with the daily ingestion of food. The weights of the various groups also paralleled these trends, and the hypophysectomized rats did not display any increase in weight, unless they received injections of the pituitary growth hormone, when considerable growth resulted.

The growth hormone did not appear to produce any noteworthy effect on normal young male rats. However, by the time the injections were started in these animals they were 60 days old and past the period of most rapid growth. The food consumption and weight gains were the same for the group which received injections of the hormone as for the untreated group.

It is reported by Nishikawara, Barnett, Maykut, Sprague, and Haist (58) that the pancreatic amylase activity of the rat was lowered by hypophysectomy. Treatment of these rats with crude saline extract

of beef pituitary gland partially restored pancreatic amylase activity. This restoration of the pancreatic activity was similar to the results obtained in serum amylase levels when growth hormone was given to the hypophysectomized animals.

XII. Effect of Codeine on Intact Rats and Mice

Other methods, besides pancreatectomy, have been used to assess the relationship between the pancreas and serum amylase levels. These have included ligation of the pancreatic ducts and the injection of parasympathomimetic drugs. Ligation of the pancreatic ducts in dogs (43) and cats (46) had resulted in elevated serum amylase values for both species. The injection of mecholyl in conjunction with eserine also increased the serum enzyme activity for these species of animal (43, 46). The administration of codeine and other opiates has been reported to raise the level of serum amylase in humans as well as cats and dogs (49, 50, 51, 52). The pharmacological action of these opiates is to cause a spasm of the sphincter of Oddi, which in turn produces "increased pressure in the pancreatic ducts and a temporary reflux of the enzyme into the blood stream" (49).

A similar experiment was devised to see if rats responded in an analagous manner to injection of codeine. A group of mice of the A strain was also studied. The experiments were so timed that the animals were actively digesting food and the pancreas was actively secreting its digestive enzymes. The latter condition is essential if a positive response is to be obtained (52).

Codeine phosphate was employed and two dosage levels studied, viz., the so-called tranquillizing dose of Barlow (53), at 63 mgm./kgm. body weight, and the standard analgesic dose of 175 mgm./kgm. recommended by Ercoli and Lewis (54). The level of the serum amylase was determined just prior to the subcutaneous injection of the drug and again after a lapse of three hours. Groups of six normal rats received the injections of codeine indicated above. A group of six animals served as controls

and were given subcutaneous injections of 0.5 ml. of physiological saline. The response of the animals is given in Table XIX.

It will be noted that codeine phosphate is without effect on the serum amylase at either dosage level. These findings are, of course, at variance with those reported for humans, and for cats and dogs. A possible reason for this deviation might be that the rat is without a gall-gladder. As noted by Mann (55), "the tonus of the sphincter of Oddi in the rat is almost negligible in contradistinction to animals which possess a gall-gladder". McMaster (56) has also studied extensively the role of the sphincter of Oddi in animals without gall-gladders and has reached essentially the same conclusion.

As the mouse possesses a gall-bladder, the above experiment was repeated with mice of the A strain. A group of six adult males received injections of codeine phosphate at the higher dosage level mentioned above, and a control group received injections of saline (0.2 ml.). Three hours later, the mice were killed by decapitation and all the blood was collected in order to obtain enough for the determination of amylase levels. The mice injected with codeine had a higher amylase value than the animals injected with saline; 52.8 ± 1.83 units/100 ml. as against 42.4 ± 1.67 units respectively. This difference is highly significant, ($t = 4.22$, $P < 0.01$). It would appear, therefore, that the effect of codeine is restricted to those animals possessing a gall-bladder.

The significance of these results is that species differences must be considered in attempts to localize the source or sources of serum amylase. This action of codeine has often been invoked to prove

Table XIX

Effect of Codeine on Rat Serum Amylase Levels

Time	Serum amylase in units per 100 ml. Experimental	Control
(a) Dosage: 63 mgm./kgm. of body weight		
Pre-injection	54.8 ± 1.18	54.7 ± 0.81
Post-injection	55.1 ± 1.46	53.5 ± 2.00
(b) Dosage: 175 mgm./kgm. body weight		
Pre-injection	53.3 ± 1.10	53.3 ± 0.94
Post-injection	52.0 ± 0.75	52.8 ± 2.00

Values shown are for the means of six rats (plus or minus the standard error of the mean).

the pancreatic origin of serum amylase, in whole or in part. It is evident, however, that such considerations require careful evaluation.

XIII. Effect of Diet

A. Introduction

The effect of composition of diet on serum amylase has been the subject of extensive investigations. Most workers have concluded that diet has no influence (11, 50, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69), although a few have stated that certain diets could modify the level of serum amylase (70, 71, 72, 73, 74). This discrepancy in results is associated at least in part with the lack of standardized experimental conditions. For example, human serum amylase levels were determined in the fasting state, and then one to three hours after the ingestion of a test meal identified only as high in concentration of carbohydrate, or fat, or protein (11, 64, 65, 75). It has also been reported that fasting is without effect on serum amylase, although here the period is limited to 24 hours generally (11, 59, 64, 65). However, it has been shown by Flock and Bollman (76) that fasting produced decreases in the level of the enzyme in rats. It will be recalled from Table V above, that it required three days without food to lower the values to a highly significant degree.

Enzymic adaptation by bacteria in response to alterations in nutritional environment has been a well-recognized phenomenon since the work of Wortmann (77). It is essential that, before such changes become fully manifested, the organism, either bacterial or animal, be given sufficient time to adjust itself to the altered nutritional milieu. For example, Grossman, Greengard, and Ivy (78) found that diets high in carbohydrate or protein produced marked changes in some of the pancreatic enzymes of the rat after 21 days. Previous work has shown that

both the alkaline phosphatase and the lipase of rat serum respond to changes in the nature of the diet (31, 33, 79, 80), and that in this respect the amount and nature of the dietary fat was the most important factor. Squires (73),in a study of certain native African tribes, found that there was a parallelism between salivary amylase activity and established levels of carbohydrate consumption. He also noted a significant correlation between levels of the enzyme in the serum and the saliva (72).

It appeared from a survey of the previous work that rigorous controls should be maintained in an attempt to study the influence of various dietary factors on rat serum amylase. These should include: the age and weight of the animals, diets of uniform composition and purity, and a continuation of the dietary regimen for a period of time sufficient to allow for enzymic adaptation.

B. Experimental

Adult male rats of uniform age and weight within each group were used. The animals were housed with individual cages and allowed ground Purina fox checkers and water ad libitum for at least a week. They were then fasted for three days (81) and placed on the experimental dietary regimen for periods of at least 28 days. With each series of experiments a group of control animals was maintained throughout on Purina fox checkers as a further control. Serum amylase determinations were made at weekly intervals according to the procedure previously outlined. Body weights were recorded at the same time as blood was collected. Daily records of food consumption were also maintained.

Other than the control regimen of ground checkers, each diet was synthetic. These diets contained either corn starch or sucrose, casein, and fat in the form of Crisco, in the proportions indicated below, as well as 4% of McCollum's Salt Mixture (82). Each diet was supplemented by 5 mgm. thiamine hydrochloride, 5 mgm. pyridoxine hydrochloride, 5 mgm. nicotinamide, 20 mgm. calcium pantothenate, 10 mgm. riboflavin, 1 gm. choline chloride, 1.2 mgm. Vitamin A acetate, 0.14 mgm. calciferol, and 120 mgm. of alpha-tocopherol per kilogram of diet. Because thiamine has been found to be unstable in synthetic diets (83), each diet was supplemented with 500 mgm. ascorbic acid per kilogram of diet to act as an antioxidant.

C. Diets Studied

1. High Sucrose, High Starch, High Protein and High Fat Diets

The first two diets contained sucrose or corn starch to the extent of 70%, and in addition 18% casein, 8% fat, and minerals and vitamins as above. The high protein diet contained 66% casein, 22% sucrose, and 8% fat. The fourth diet, high in fat, contained 45% Crisco, 33% sucrose, and 18% casein. The pre-dietary values for serum amylase, body weights, and daily food consumption are given in Table XX. In addition, these values are indicated at weekly intervals during the four week ad libitum experimental feeding period, as well as for two weeks after the animals were restored to the control diet of ground checkers.

One might have expected that the high starch diet would have resulted in some significant alteration of amylase levels. However, although the serum amylase tended to be higher with the high starch than the high sucrose diet, in neither case were levels significantly altered from the control values. Consumption values for the two carbohydrate diets were markedly less than for the control diets, but not to a sufficient extent to affect values through inanition (cf. Table VI). It should also be remembered that the synthetic diets were free of crude fibre, and that the differences in consumption were not strictly comparable.

The high protein diet in four weeks effected a highly significant diminution in rat serum amylase levels ($P < 0.01$). Following return to the control diet, increased consumption was accompanied by elevation of enzyme values to normal. The results for the high fat diet were surprising and the highly significant increase in enzyme activity ($P < 0.01$) was quite unexpected. These findings are strengthened

by the fact that the above experiment was repeated twice more and the results did not differ materially from those in Table XX.

Studies were made of the amylase content of the various tissues of the rat which had subsisted on the various dietary regimens discussed above. The results of these studies, which are given below, are in substantial agreement with those of Grossman et al (78) in the case of pancreatic amylase and also provide a rationale for some of the results of Table XX.

Table XX

Effect of High Carbohydrate, High Protein and High Fat Diets on Rat Serum Amylase (A) in Units/100 Ml. Body Weight (W) in Gm. and Food Consumption (C) in Gm./Day. Mean of Six Rats

Time	Control			High Sucrose			High Starch			High Protein			High Fat		
	A	W	C	A	W	C	A	W	C	A	W	C	A	W	C
Pre-diet	55.4	382	22	58.3	295	21	61.9	297	20	60.4	320	21	57.4	303	21
Diet															
Week 1	61.0	390	21	61.8	300	16	66.7	310	19	49.9	326	15	72.5	328	15
2	59.3	397	22	58.2	311	15	61.4	312	16	46.3	336	15	67.2	349	13
3	57.9	403	20	56.8	318	14	60.5	323	16	45.1	345	14	67.9	365	12
4	58.9	404	21	57.0	326	14	59.3	331	16	49.8	350	12	67.6	378	12
Post-diet															
Week 1	58.0	412	20	58.9	335	16	61.0	347	17	61.9	355	19	59.9	380	14
2	63.2	413	23	59.2	339	23	60.5	346	20	64.2	366	22	59.1	380	19

2. Diets with Varying Concentrations of Fat

Because fat had such a significant effect on the levels of rat serum amylase, it was decided to investigate the effect of fat at different dietary concentrations, to see if the degree of response by the enzyme was proportional to the fat content of the diet. Accordingly a series of diets was prepared, which contained fat at levels of 0, 5, 15, 25, 45, and 60%. All these diets contained 18% casein, 4% salts, and the usual supplements of vitamins. The balance of the diet was made up with sucrose in each case. The results of this study are reported in Table XXI.

It will be noted in Table XXI that the fat-free diet resulted in the lowest amylase levels. There was a gradual increase in enzyme activity as the dietary fat concentration was raised from 0 to 45%. After four weeks the group of rats subsisting on the 60% fat diet, manifested amylase values below those for the group which were fed the 45% Crisco diet. The highest concentration of dietary fat resulted in a generally lower food consumption over the experimental period and so other metabolic and physiological factors became operative. For example, over the four week period the group of rats maintained on the 45% Crisco diet showed a mean weight gain of 75 gm., while the group on the 60% fat diet gained but 44 gm.

Statistical treatment of Table XXI shows a highly significant correlation between the amount of fat in the diet and the level of the serum enzyme ($r \simeq 0.989$, $t \simeq 3.56$, $P < 0.01$).

Table XXI

Effect of Dietary Fat Level on Rat Serum Amylase (A) in Units/100 ml., Body Weight (W) in Gm., and Food Consumption (C) in Gm./Day

	Control			0% Fat			5% Fat			15% Fat			25% Fat			45% Fat			60% Fat		
	A	W	C	A	W	C	A	W	C	A	W	C	A	W	C	A	W	C	A	W	C
Pre-diet	62.2	345	21	57.7	340	22	56.8	355	23	57.5	363	21	54.7	320	21	57.4	303	21	51.2	346	20
Diet																					
Week 1	66.4	362	21	55.7	348	15	57.8	360	17	59.8	382	16	63.9	353	12	72.5	328	15	63.8	360	11
2	62.8	377	20	54.1	359	17	58.4	374	16	61.6	392	13	61.4	358	14	67.2	349	13	59.5	369	10
3	62.7	372	21	60.0	362	14	59.4	377	16	63.6	396	12	64.5	367	11	67.9	365	10	64.2	379	10
4	66.9	391	20	54.4	374	14	59.7	390	14	62.9	408	14	63.3	378	14	67.6	378	12	59.8	390	12
Post Diet	57.7	401	21	59.7	382	22	57.6	395	22	58.3	412	18	60.6	384	19	59.2	380	19	57.6	388	16

Each value shown is the mean of six rats.

3. Diets Lacking in Available Carbohydrate

A group of animals was maintained for a period of four weeks on a synthetic diet which contained no carbohydrate. This regimen consisted of the usual vitamins and salts, 11% fat and 85% protein, so it was in fact a carbohydrate-free, high protein diet. Two other diets were used in this experiment, in which the readily digestible carbohydrate (corn starch or sucrose) was replaced by the same amount (70%) of cellulose (not digestible) or uncooked potato starch (not readily digestible (84, 85, 86, 87)).

The data for serum amylase presented in Table XXII show that the three experimental diets had produced no effect in one week that was significantly different from the pre-experimental or control values. However, by the end of the second week amylase levels had fallen in the three experimental groups. These decreases in enzyme activity were all highly significant ($P < 0.01$) and cellulose produced the greatest fall in serum amylase. The cellulose diet also produced a loss of weight and abnormally great consumption of food. The data listed in Table XXII for this group were similar to those noted previously with the fasted animals (cf. Table V). On both the carbohydrate-free and the raw potato starch diet good growth was maintained. The lack of readily digestible carbohydrate in the latter regimen seemed to be the factor associated with the lowered amylase levels. The enzyme levels for the group which received no carbohydrate were influenced by the high protein nature of the diet (Table XX) as well as by the abnormally low consumption. It would be useless to speculate about what effect, if any, may be ascribed to the absence of carbohydrate.

Table XXII

Effect of Diets Free of Available Carbohydrate on Rat Serum Amylase (A) in Units/100 Ml., Body Weight (W) in Gm. and Food Consumption (C) in Gm./Day. Mean of Six Rats

Time	Control			Cellulose			Raw Potato Starch			Carbohydrate Free		
	A	W	C	A	W	C	A	W	C	A	W	C
Pre-diet	60.2	373	21	63.7	334	19	58.3	341	20	61.8	328	21
Diet												
Week 1	61.0	377	21	60.2	332	18	59.2	335	15	61.8	329	14
2	59.4	379	20	42.5	297	25	49.5	337	23	51.7	333	16
3	59.3	389	22	36.1	281	29	46.0	339	20	52.9	324	13
4	57.9	384	21	36.1	273	31	44.5	343	17	49.4	342	12
Post-diet	58.9	383	21	58.4	308	24	64.7	373	24	58.5	353	18

4. Protein-free diets

Two protein-free diets were also studied. Both diets contained 11% fat, and 85% of either sucrose or corn starch. These, as well as the usual control diet of ground checkers, were fed to three groups of six rats each, and the usual experimental procedures were followed.

Some extremely interesting results are reported in Table XXIII. While the amylase values for the starch-Crisco diet more or less paralleled those for the control group, there was a very marked and highly significant decrease in enzyme levels of the group on the sucrose-Crisco diet by the end of the second week. In the case of the latter diet, since protein was lacking, and since as was noted in Table XXI that this level of fat did not alter serum amylase from the normal values, it would appear that the polysaccharide corn starch is one factor which stimulates the maintenance of "normal" serum amylase activity.

From these studies it is apparent that the composition of the diet has a definite influence on the level of the serum amylase in the rat. This enzyme is not restricted to any one tissue or organ, and consequently its level in the blood may be the summation of the contributions of a number of sources. At lease some of the tissues may be expected to vary in the production of amylase in accordance with the altered nutritional environment of the animals. Hence to evaluate fully the effect of diet on serum amylase activity it has been considered necessary to study the relationship between alterations

Table XXIII

Effect of the Nature of the Carbohydrate in Protein Free Diets on Rat Serum Amylase (A) in Units/100 Ml., Body Weight (W) in Gm. and Food Consumption in Gm.Day. Mean of Six Rats

Time	Control			Sucrose and Crisco Diet			Starch and Crisco Diet		
	A	W	C	A	W	C	A	W	C
Pre-diet	55.5	332	22	55.9	339	16	54.0	343	21
Diet									
Week 1	54.3	347	20	54.9	313	15	63.8	317	13
2	57.1	354	23	49.9	294	11	54.4	292	13
3	53.6	361	20	46.9	277	11	54.7	273	11
4	57.5	361	21	46.4	260	13	55.6	257	9
5	59.3	364	20	47.2	245	12	55.8	239	9
6	57.9	375	21	44.1	233	11	55.6	228	9
Post-diet									
Week 1	54.9	379	22	61.1	299	27	52.4	295	20
2	52.9	383	19	52.3	338	22	52.7	328	21

in serum amylase in response to varied dietary regimens and variations in the levels of certain tissue amylases. This is the subject of the succeeding section.

INVESTIGATION OF TISSUE AMYLASES

I. Introduction

The earliest recorded investigations of comparative tissue amylase assay are those of Carlson and Luckhardt in 1908 (88). These studies were confined to the body fluids but a large number of animals was examined. In general, the level of amylase in decreasing order of activity was: serum, thoracic lymph, neck and limb lymph, pericardial fluid, cerebrospinal fluid. Hirata (89) made a similar investigation of tissues in man, and the dog, cat, rabbit, guinea pig, hen, fish, frog,and rat, and reported the following levels of activity in descending order: pancreas, serum, liver, kidney, muscle, and spleen. Subsequently the presence of amylolytic activity was also reported in the adipose tissue of the dog, rat, rabbit, mouse, as well as man (90, 91, 92), and also in lung tissue of several species (93).

A few recent studies of the distribution of tissue amylase activity have also been made. Yamagata (94) reported the following levels in decreasing order of activity in the rabbit: pancreas, kidney, muscle, spleen, and liver. Okamoto (93) has made an extensive study including the rabbit, rat, guinea pig, bull frog, chicken and pigeon, and has found the following order of activity in decreasing levels: kidney, liver, lung, skin, muscle, and brain. Okamoto also found that the rat and the guinea pig possessed the highest amylolytic activity in all the tissues investigated except the liver. Roe, Smith, and Treadwell (48) place the order of amylase activity in the following decreasing order for the rat: pancreas, liver, duodenum, jejunum, kidney, heart, spleen, muscle.

It will be noted that the various investigators did not find the same order of activity but that some variation was found. While some of the divergent results might be attributed to species differences, it is possible that the differing methods of enzyme assay were also responsible for the variations in the order of activity. This would be particularly true for liver and muscle amylases if a dextrinogenic technique was used in some instances and a saccharogenic method in others, unless the latter included a phosphorylase inhibitor.

II. Normal Values

Since the reported levels and order of tissue amylase activity varied considerably, it was felt necessary to redetermine the amylolytic content of the various organs of the rat. In preliminary studies, the animals were killed by decapitation, and then certain tissues were removed for assay. The recovery of the parotid glands proved difficult under this procedure. An alternative technique was adopted in which the animals were sacrificed by a lethal dose of Nembutal. As this chemical occasionally inhibits or suppresses enzyme activity, it was necessary to establish if it had any influence on tissue amylase content or its activity.

In the preliminary investigation, the tissue homogenates of six decapitated animals were assayed for amylase activity. The results of these determinations were compared to enzyme values obtained from rats which had been killed by an overdose of Nembutal. The mean amylase levels for each group were as follows, (with the values for the Nembutal treated animals shown in parenthesis): serum 58.2, (60.4); pancreas 56,000 (53,000); liver 12.3 (13.7); spleen nil, (nil); heart nil (nil), and depot fat 20, (24). All these values are in units of amylase activity per 100 gm. of wet tissue. These results indicated that the effect of Nembutal on tissue amylase activity was insignificant.

The technique for amylase assay in tissues was as follows: after death, the various organs were removed as quickly as possible and placed in the cold. The tissue was weighed and then homogenized in a Potter Elvehjem glass homogenizer with ice cold saline. The whole homogenates were used for amylase estimations. It was necessary to run preliminary trials with the homogenates to establish a suitable enzyme

dilution. In general, the final enzyme concentration was adjusted so that its amylase content would be similar to that of a serum specimen, i.e. 50 to 60 amylase units per 100 ml. of enzyme homogenate. The results of these studies were reported as units of enzyme activity per 100 gm. of wet tissue.

The actual methods employed for amylase assay followed that detailed for the estimation of serum amylase activity. In each instance the enzyme preparation was used for the blank reading and did not vary from the blank reading found with dilute serum. The pH optimum for each tissue amylase was determined. In every instance the pH optimum was found to be 7.0, which corresponded to that reported for the serum enzyme. The results of these investigations will be found in Table XXIV.

It will be noted that the highest level of activity was that of the parotid glands, followed by the pancreas, and then the intestinal mucosa. These tissues had activity in excess of that of the blood serum. All the other tissues studied possessed activities lower than that of the serum, although the lung, depot fat, kidney, and liver still retained considerable amylolytic action. The spleen, heart, and brain did not display any amylolytic activity.

A few general remarks might be of interest. The very high level of the parotid tissue was unexpected, for it was assumed that it would be of the same order as that of the pancreas. No mention of this extremely high value has been found in the literature. The amylase of the intestinal mucosa might be indigenous to the gut or it could represent trapped pancreatic secretion. The association between pancreas and small intestine in the rat is intimate and attempts to strip all the

Table XXIV

Amylolytic Activity of Rat Tissues

Tissue	Amylase activity units/100 gm.wet tissue	Range
Parotid glands	107,400 ± 25,000	77,000 - 151,000
Pancreas	54,700 ± 3,850	30,000 - 80,000
Intestinal mucosa	428 ± 195	300 - 800
Serum	61.6 ± 3.4	57 - 62
Lung	37.7 ± 2.4	13 - 87
Depot fat (peri-renal)	30.0 ± 2.0	13 - 40
Kidney	21.1 ± 2.3	16 - 27
Liver	13.1 ± 1.8	10 - 15
Testicle	4.5 ± 0.5	0 - 9.7
Muscle (thigh)	2.7 ± 0.2	0 - 5.9
Spleen	Nil	-
Heart	Nil	-
Brain	Nil	-

Each recorded value is the mean for ten rats (plus or minus the standard error of the mean).

pancreatic tissue from the gut were not wholly successful. By virtue of its high amylase content the slightest trace of pancreatic tissue in whole gut homogenates would influence the final results disproportionately. For this reason intestinal mucosa was studied in place of the intact gut. The first 20 cm. of gut was thoroughly flushed with water, then dried on filter paper. It was next everted on a glass rod and the mucosa was scraped onto a watch glass, weighed, and homogenized.

The presence of an amylolytic enzyme in the lung and depot fat is puzzling and indicates that this enzyme must have some other role than a purely digestive function. It will be recalled that the lung, liver, and kidney are richly vascularized so that some of the enzymic activity could be due to residual blood. The depot fat, however, has a poor blood supply and its enzyme activity cannot be attributed to this source.

III. Effects of Starvation and Pancreatectomy

The level of the tissue amylase activity was also studied in rats which had been starved for seven days and in a group of animals which had been pancreatectomized for 330 days. For purposes of comparison, the normal values recorded above are included in Table XXV. In addition to the serum, the level of the parotid gland activity, the pancreas, and the intestinal mucosa, were studied.

As the data in Table XXV indicate, starvation was accompanied by highly significant declines in amylase of serum, pancreas, and intestinal mucosa ($P < 0.01$ for all). The parotid amylase activity was not changed by starvation. It is possible that some of the decreased serum activity might be due to the drop in the level of the pancreatic enzyme. There is also the possibility that the disappearance of the depot fat resulted in decreased serum amylase levels. The level of the intestinal mucosa is very much lower but whether this is due to the decreased pancreatic activity is difficult to say.

Pancreatectomy has resulted in highly significant decreases in both the serum and mucosal amylase levels ($P < 0.01$ for both). Again the parotid gland amylase remained unaltered. The fall in intestinal mucosa amylase could have been due to the removal of the pancreas. Roe et al (48) reported a similar finding in their experiments with depancreatized rats. These workers, using a newly developed technique, were able to achieve complete pancreatectomy. They felt that the residual amylase in the small intestine could have been derived from the salivary glands. From the studies reported here, it is apparent that some other factor or factors besides the pancreas is responsible for the marked decline in serum amylase with extended fasting.

Table XXV

Tissue Amylase Levels in Starved and Depancreatized Rats

Tissue	Normal Rats	Starved Rats	Pancreatectomized Rats
Serum	61.6 ± 3.4	21.9 ± 2.7	52.2 ± 3.8
Parotids	107,400 ± 25,000	164,800 ± 31,000	127,200 ± 29,500
Pancreas	54,700 ± 3,850	10,050 ± 1,750	-
Mucosa	428 ± 95	14.3 ± 1.1	133 ± 42

Values shown are the mean of ten animals (plus or minus the standard error of the mean).

Amylase activity is per 100 gm. of wet tissue.

Animals were starved for seven days.

IV. Effect of Composition of Diet

Since the serum amylase level underwent changes with different diets, it was decided to see whether these changes were also reflected by altered levels of tissue amylases. Four groups of six rats each were maintained on either a high sucrose, high starch, high protein, or high fat diet respectively for 28 days. The composition of these diets was identical to the ones already mentioned. On the 28th day of the diet, the animals were sacrificed and the parotid glands, serum, pancreas, intestinal mucosa, and depot fat were assayed for amylase activity. The results are presented in Table XXVI.

The effect of these diets on the serum amylase has already been reported and discussed above. Briefly, the high sucrose and the high starch diet were without effect, while the high fat diet increased, and the high protein diet depressed serum amylase values. In studying the other tissues, one again notices the stability of the parotid tissue with respect to its level of amylase activity. The pancreas, however, had undergone a highly significant change with both the high sucrose and the high starch diets ($P < 0.01$ for both). This increased level of the pancreas has previously been reported for high starch diets by Grossman et al (78) and Hirata (89). Although the amylase activity of the pancreas had increased, there was no corresponding increase in the serum. The high protein and the high fat diet have not affected the level of the pancreatic amylase.

The changes in amylase activity of the intestinal mucosa, in the main paralleled those of the pancreas, with highly significant increases ($P < 0.01$) for the high starch and high sucrose regimens. There was a

Table XXVI

Effect of Diet on Tissue Amylase Levels

Diet	Serum	Parotid glands	Pancreas	Mucosa	Depot Fat
Normal	59.7 ± 1.3	124,000 ± 24,500	51,300 ± 3,450	437 ± 43	25.5 ± 1.5
High Sucrose	60.3 ± 3.6	118,000 ± 27,800	77,600 ± 4,620	653 ± 105	19.8 ± 1.8
High Starch	62.8 ± 2.5	118,000 ± 29,200	90,000 ± 5,210	824 ± 87	21.6 ± 2.2
High Protein	51.7 ± 1.6	103,000 ± 26,300	52,300 ± 4,380	324 ± 61	9.4 ± 0.08
High Fat	66.5 ± 2.0	107,000 ± 19,600	57,000 ± 3,760	848 ± 78	76.1 ± 4.7

Values reported in units per 100 gm. wet tissue, and represent the mean of six animals (plus or minus the standard error of the mean).

significant decline ($P < 0.05$) on the high protein diet and a highly significant increase ($P < 0.01$) on the high fat diet.

The effect of these diets on depot fat amylase is probably the most interesting. The high starch and high sucrose diets were without effect, but the high protein diet resulted in a highly significant decline ($P < 0.01$) and the high fat diet in a highly significant increase ($P < 0.01$) in the amylase of depot fat. These changes are paralleled by similar changes in the serum, and a highly significant correlation coefficient was found ($r = 0.784$, $P < 0.01$) between the levels of the amylase activity in serum and depot fat, for those groups existing on the stock diet and those on the high fat diet. It is quite possible therefore that one of the factors which affected the level of the serum amylase might have been the amylolytic activity of the adipose tissue. In the high fat diet, although the increase in depot fat amylase activity is only threefold, it should be remembered that the total increase in depot fat (especially in the mesenteries) is at least four to five fold, and so the increase is probably 12 to 15 fold, if not greater. The actual role of amylase in depot fat is unknown and it presents an intriguing subject for further study. Mirski (91) has found considerable glycogen reserves in rat depot fat. It could be that the amylase is involved in its formation or disappearance.

The high protein diet caused highly significant decreases in both the serum and depot fat enzyme activity. It will be recalled that starvation or reduction of food intake brought about decreased serum amylase levels. Since this will also cause the adipose tissue to dis-

appear, it may be that this is part of the explanation for the decreased serum amylase level in inanition.

The amylase value of the intestinal mucosa also ran parallel to the depot fat level and the possibility exists that the mucosal amylase might be partially derived from this source.

V. Effect of Ethionine

The destruction of acinar tissue in rat pancreas by repeated doses of ethionine has been reported by several investigators (95, 96, 97, 98). Bollag and Gallico (97) observed that this destruction was accompanied by marked decrease of pancreatic amylase, and that this decrease was proportional to the degree of acinar damage. Almeida and Grossman (99) found similar changes in cats, dogs, and monkeys. These workers noted a transient fall in the serum amylase of the dog after ethionine administration. This decrease was later replaced with a sharp rise coincident with the development of pancreatitis.

A series of experiments was undertake to evaluate the effect of intraperitoneal injections of ethionine on rat serum amylase. In the course of these experiments, it was noted that the ethionine produced a state of intense anorexia. This condition itself will result in significantly lowered levels of rat serum amylase. In order to circumvent this situation, the experiment was redesigned and a control group with paired feeding was also included.

A group of six male adult rats, housed in individual cages, was fed a diet of ground fox checkers and water ad libitum. After one week, intraperitoneal injections of 100 mgm. ethionine in 4 ml. of water were instituted and continued for a period of 14 days. Blood for enzyme estimation was collected from the tail, just before the initial injection and again on the seventh and fourteenth day of the injections. After the final injection, the animals were sacrificed and the pancreas, the parotid glands, and the first 20 cm. of gut were removed for amylase determination. Daily records of food consumption were kept

during the experiment. A second group of six animals served as controls and a paired feeding regimen was followed. This group received 4 ml. of physiological saline in lieu of the ethionine solution, otherwise the same experimental protocol obtained throughout the experiment.

The effect of the ethionine injection on rat serum amylase is given in Table XXVII. At the start of the experiment no significant difference existed between the serum amylase levels of the experimental and the control animals. At the end of seven days, both groups have shown highly significant decreases from the initial values although that in the group receiving the ethionine was more marked, (experimental, $t = 9.6$, control, $t = 12.1$, $P < 0.01$ for both). This trend continued and was even more noticeable at the end of the 14th day of the ethionine injection (experimental, $t = 17.7$, control, $t = 7.0$, $P < 0.01$ for both).

Insofar as the control group was concerned, the decline in serum amylase may be attributed to the reduced food intake, an effect noted above. However, in addition to this phenomenon, there was a highly significant difference between the experimental and the control group at both the seventh and the fourteenth day of treatment, with the ethionine treated rats showing the lower level (7th day, $t = 3.21$, 14th day, $t = 4.97$, $P < 0.01$ for both). This difference presumably is brought about by some factor other than food. Consideration of Table XXVIII, which gives the results of the tissue amylase determinations, reveals one possible explanation.

Treatment with ethionine has brought about highly significant decreases in both the pancreatic ($t = 4.63$, $P < 0.01$) and the mucosal ($t = 3.49$, $P < 0.01$) enzyme levels while that of the parotid glands remains

Table XXVII

Ethionine Injection and Serum Amylase Activity

Day of treatment	Serum amylase Units/100 ml.	Body weight in gm.	Food consumption gm./diem
Pre-injection	62.0 ± 2.1	263 ± 6.5	20
	59.3 ± 0.9	270 ± 3.2	20
7th day	24.4 ± 3.2	232	2
	36.3 ± 1.7	242	2
14th day	14.1 ± 1.7	175 ± 9.3	0
	27.8 ± 4.3	196 ± 4.8	0

Upper row of figures refer to experimental animals receiving ethionine.

Lower row of figures refer to control animals receiving saline.

Values shown are the mean of six animals (plus or minus the standard error of the mean).

Table XXVIII

Changes in Tissue Amylase Levels after Ethionine Injection

Tissue	Experimental units/100 gm. wet tissue	Control units/100 gm. wet tissue
Serum	14.1 ± 1.7	27.8 ± 4.3
Pancreas	584 ± 220	41,460 ± 2770
Intestinal Mucosa	20.6 ± 5.0	67.0 ± 28
Parotid glands	101,100 ± 28,000	107,600 ± 34,000

Values shown are the mean of six animals (plus or minus the standard error of the mean).

unchanged. Since there is also a highly significant difference between the serum amylase in the experimental and control groups, it is possible that this difference might be due in part to changes in the enzyme level of the pancreas and the intestinal mucosa, provided that the latter is indigenous to the gut.

DISCUSSION

Some of the serum amylase activity of the rat is derived from the pancreas. The effect of pancreatectomy, ethionine injection, and starvation, all support this statement. Nevertheless, an appreciable amount of the enzyme is extra-pancreatic in origin.

It may be that the parotid glands are also involved. Under the experimental conditions of this study, the very high enzyme activity of these glands remained quite stable and did not appear to contribute to the maintenance of serum amylase. It is suggested that extirpation of the parotids would be necessary to settle this problem. In this respect, an especially illuminating experiment would be the administration of ethionine to parotidectomized rats, or perhaps, the study of parotidectomized, pancreatectomized animals. These rats would be free of the two major sources of tissue amylase activity, hence if amylolytic activity still remained in the blood, a further source must be sought.

At this point, a slight digression would be of interest. The dog is an animal which does not possess salivary amylase activity. Reid, Quigley and Meyers (47) reported that depancreatized dogs had normal serum amylase levels provided that insulin was administered. The experiments of Flock and Bollman (41, 42) indicated that liver has no effect on the plasma enzyme level in canines. Consequently, the dog has a source of serum amylase, which is distinct from the pancreas, liver, and salivary glands.

Any tissue that is a potential source of the serum enzyme must possess amylolytic activity itself. The adipose tissue of man, rabbits, mice, and rats, as well as that of canines, all display amylase

activity. The depot fat of dogs, rats, and mice possess considerable glycogen reserves. Mirski (91) has reported that the breakdown of depot fat glycogen in rats by amylolytic action far exceeds that due to phosphorolysis. The adipose tissue appears to contain a large number of glycolytic enzymes but they have been the object of little study. It is possible that this tissue may contribute to serum amylase activity. In the rat, fasting was accompanied by decreased levels of serum and pancreatic amylase. In addition the depot fat disappeared. Furthermore, high fat diets resulted in elevated depot fat activity as well as increased serum levels. These particular studies point to another possible source of rat serum amylase, viz., depot fat. However, further study is indicated before any categorical claims are made.

The liver does not appear to be a source of serum amylase. The low level of enzyme activity in the tissue itself indicates that it has little amylolytic function.

Finally, the intestinal mucosa requires consideration. Unfortunately this is a difficult tissue to study. An overzealous approach to the preparation of homogenates will result in considerable loss of mucosa. On the other hand, a cautious technique would mean the retention of considerable pancreatic secretion, with misleading results in enzyme assay. At the present time, it is not possible to make an unequivocal statement that a native mucosal amylase exists, and until this is so, speculation should be avoided.

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